10/540094
Rec'd PCT/PTO 20 JUN 2005

The Patent Office

INVESTOR IN PEOPLE

The Patent Office
Concept House
Cardiff Road
Newport
South Wales
NP10 8QQ

PRIORITY DOCUMENT
SUBMITTED OR TRANSMITTED IN COMPLIANCE WITH RULE 17.1(a) OR (b)

REC'D 06 FEB 2004
WIPO PCT

I, the undersigned, being an officer duly authorised in accordance with Section 74(1) and (4) of the Deregulation & Contracting Out Act 1994, to sign and issue certificates on behalf of the Comptroller-General, hereby certify that annexed hereto is a true copy of the documents as originally filed in connection with the patent application identified therein.

In accordance with the Patents (Companies Re-registration) Rules 1982, if a company named in this certificate and any accompanying documents has re-registered under the Companies Act 1980 with the same name as that with which it was registered immediately before re-registration save for the substitution as, or inclusion as, the last part of the name of the words "public limited company" or their equivalents in Welsh, references to the name of the company in this certificate and any accompanying documents shall be treated as references to the name with which it is so re-registered.

In accordance with the rules, the words "public limited company" may be replaced by p.l.c., plc, P.L.C. or PLC.

Re-registration under the Companies Act does not constitute a new legal entity but merely subjects the company to certain additional company law rules.

Signed Stephen Hordley

Dated 20 January 2004

BEST AVAILABLE COPY

Patents Form 1/77

Patents Act 1977
(Rule 16)

THE PATENT OFFICE
20 DEC 2002
DELIVERED BY HAND

The Patent Office

24DEC02 E772907-6 D00056
P01/7700 0.00-0229838.8

1/77

Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

The Patent Office

Cardiff Road
Newport
South Wales
NP9 1RH

1. Your reference — P34080GB/KVC

2. Patent application number *(The Patent Office will fill in this part)* — 0229838.8 — 20 DEC 2002

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

   Mars UK Limited
   3D Dundee Road, Slough
   Berkshire SL1 4LG

   Patents ADP number *(if you know it)* — 07478787001

   If the applicant is a corporate body, give the country/state of its incorporation — United Kingdom

4. Title of the invention

   Multi-Component Meal

5. Name of your agent *(if you have one)*

   "Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

   Kilburn & Strode
   20 Red Lion Street
   London
   WC1R 4PJ

   Patents ADP number *(if you know it)* — 125001

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing *(day / month / year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*
   a) any applicant named in part 3 is not an inventor, or
   b) there is an inventor who is not named as an applicant, or
   c) any named applicant is a corporate body.
   See note (d))

   YES

Patents Form 1/77

9. Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document

Continuation sheets of this form

Description 62

Claim *(s)* 2

Abstract

Drawing *(s)* 16 + 16 SV

10. If you are also filing any of the following, state how many against each item.

Priority documents

Translations of priority documents

Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*

Request for preliminary examination and search *(Patents Form 9/77)*

Request for substantive examination *(Patents Form 10/77)*

Any other documents *(please specify)*

11. I/We request the grant of a patent on the basis of this appli

Signature Kilburn & Strode

Date 20/12/2(

12. Name and daytime telephone number of person to contact in the United Kingdom

Kristina Cornish
Tel: 020 7539 4200

Warning

After an application for a patent has been filed, the Comptroller of the Patent Office will consider whether publi or communication of the invention should be prohibited or restricted under Section 22 of the Patents Act 1977. will be informed if it is necessary to prohibit or restrict your invention in this way. Furthermore, if you live in t United Kingdom, Section 23 of the Patents Act 1977 stops you from applying for a patent abroad without first g written permission from the Patent Office unless an application has been filed at least 6 weeks beforehand in th United Kingdom for a patent for the same invention and either no direction prohibiting publication or communication has been given, or any such direction has been revoked.

Notes

a) *If you need help to fill in this form or you have any questions, please contact the Patent Office on 0645 5005*

b) *Write your answers in capital letters using black ink or you may type them.*

c) *If there is not enough space for all the relevant details on any part of this form, please continue on a separat sheet of paper and write "see continuation sheet" in the relevant part(s). Any continuation sheet should be attached to this form.*

d) *If you have answered 'Yes' Patents Form 7/77 will need to be filed.*

e) *Once you have filled in the form you must remember to sign and date it.*

f) *For details of the fee and ways to pay please contact the Patent Office.*

Multi-Component Meal

The present invention relates to an animal's multi-component foodstuff which enables the animal to optimise the macronutrient content of its diet. The invention also relates to food compositions for use in such a foodstuff; and to use of the multi-component foodstuff in animal health benefits.

This invention is based on the observation that when consuming food, animals are attempting to reach a target intake of each of the three macronutrients (protein, carbohydrate, fat) within a given time period.

This invention addresses the problem of providing palatable foods for animals, while also offering health benefits to the animal and increased acceptance/increased enjoyment in feeding.

Historically, the majority of research work on "palatability" (the relative acceptance of and preference for different foods) has concentrated on optimising the organoleptical qualities of the food. The assumption has been that the acceptability of a food and preference for one food over another are primarily driven by the taste and texture of the food. The assumption has been that as long as the nutrient content of foods exceed the minimum requirements of the animal, it will not discriminate between diets of differing nutrient profile unless there is an indirect effect on the taste or texture of the diets. This invention is based on data that demonstrates that this is not the case. When given the opportunity to do so, by provision of foods of different macronutrient contents, the animals will select between these foods so as to regulate their consumption of each macronutrient in order to reach an optimum ratio.

The present invention has identified that there is a limit to the amount of carbohydrate that animals are willing to consume. In order not to do so, they are prepared to sacrifice their calorie and/or protein intake. However, the short and long-term effects

of sacrificing macronutrient content of an animal's diet are not beneficial.

Different animals, including different species and breeds of animal will have different optimum macronutrient content for their diets. Furthermore, an individual animal is likely to have a changing optimum macronutrient content of their diet, depending on factors such as life stage, sex, sexual activity, illness, seasonal variation, environment, stress levels etc.

Accordingly, the present invention provides a multi-component foodstuff comprising two or more compartmentalised food compositions of which at least two of the compositions differ in their content of at least two selected from the group consisting of fat, protein and carbohydrate.

By the term compartmentalised it is meant that the two or more food compositons are not mixed. They may be provided on or in different containers, such as a bowl, plate, packaging. The containers may or may not be sealed. The multi-component meal comprising the two or more food compositions may be provided in unlimited quantities to the animal.

The compositions themselves may be a food product in their own right. In particular they may be a food product for a companion animal. Each may be a dry, semi-moist or a moist (wet) product. Wet food includes food that is usually sold in a container, such as a tin, pouch or tray and has a moisture content of 70% to 90%. Dry food includes food having a similar composition but with 5% to 15% moisture, often presented as small biscuit – like kibbles. Semi-moist food includes food having a moisture content of from above 15% up to 70%. The amount of moisture in any product may influence the type of packaging that can be used or is required. The food product, of any moisture level may be ready-to-eat.

The compositions encompass any product that an animal consumes in its diet. Thus, the compositions may include the standard food products as well as food products for companion animals, such as food snacks (for example snack bars, cereal bars, snacks, treats, biscuits and sweet products). The composition may be a cooked product. It may incorporate meat or animal-derived material (such as beef, chicken, turkey, lamb, fish, blood plasma, marrowbone, etc or one or more thereof). Alternatively the composition may be meat-free (preferably including a meat substitute such as soya, maize gluten or a soya product) in order to provide protein. The composition may contain additional protein sources such as soya protein concentrate, milk, protein, gluten, etc. The composition may also contain starch, such as one or more grains (e.g. wheat, corn, rice, oats, barley, etc) or may be starch-free. The composition may incorporate or be a gelatinised starch matrix. The composition may incorporate one or more types of fibre such as sugar beet pulp, chicory pulp, chicory, coconut endosperm fibre, wheat fibre etc. Dairy products, such as those incorporating a cream or a cheese sauce, may be suitable. The composition can also be newly designed products currently not available. The most suitable composition may be a pet food product as described herein which is sold as a pet food, in particular a pet food for a domestic dog or a domestic cat. It may be convenient to provide the compositions in a dry format, such as dried ready-to-eat cereal products (often referred to as kibbles).

It is important to allow the animals to freely self-select.

The compositions in the first aspect of the invention may be nutritionally complete either alone or in combination and as such, the practice of the invention may provide a suitable nutritionally complete diet for the animal.

In accordance with the first aspect of the invention the two or more food compositions may differ in their content of at least two of fat, protein and carbohydrate by at least 1% on an energy ratio basis (protein:energy ratio, or fat:energy ratio or carbohydrate:energy ratio).

In the foodstuff according to the first aspect of the invention, the difference in fat content of at least two components may be from 1% to 40% on a fat:energy ratio. The difference in protein content of at least two components may be from 1% to 40% on a protein:energy ratio. The difference in carbohydrate of at least two components in accordance with the first aspect of the invention may be from 1% to 40% on a carbohydrate:energy ratio.

The two or more different food compositions may be enriched in one or more macronutrient i.e. fat, protein and/or carbohydrate. Any composition providing an enriched source of fat preferably comprises from 20% to 90% fat on a fat:energy ratio basis. Preferably, such a composition may comprise from 50 to 75% fat on a fat:energy ratio basis.

Any composition providing an enriched source of protein preferably comprises from 18 to 90% protein on a protein:energy ratio preferably such a composition comprises from 50 to 75% protein on a protein:energy ratio.

Any composition providing an enriched source of carbohydrate preferably comprises from 20 to 90% carbohydrate on a carbohydrate:energy ratio. Preferably, such a composition comprises from 20 to 50% carbohydrate on a carbohydrate:energy ratio.

All ratios described herein are determined as the number of the calories coming from the fat, protein or carbohydrate as a % of the total calories in the composition.

The multi-component foodstuff of the invention is preferably for a companion animal, such as a dog or cat, particularly the domestic dog (*Canis domesticus*) or the domestic cat (*Felis domesticus*). It may also be for a human, an equine animal such as the horse, or other animal including fish and birds.

Optionally, at least one composition of the multi-component foodstuff comprises a dried ready-to-eat cereal product. Two or more compositions may comprise such dried ready-to-eat cereal products. Alternatively, together with one or more dried ready-to-eat cereal products, there may be a wet or semi-moist product.

The food compositions are preferably packaged. In this way the consumer is able to identify, from the packaging, the ingredients and macronutrient content of the product and confirm that it is suitable for the particular animal in question. The packaging may be metal (usually in the form of a tin or flexifoil), plastic (usually in the form of a pouch or bottle), paper or card. The amount of moisture in any product may influence the type of packaging, which can be used or is required. The foodstuff may be available as a "kit" or "pack" wherein the different food compositions are individually packaged and these packages are somehow joined together, for example in a box and/or with overarching packaging for the two or more packages of food compositions.

Thus, the food compositions of the first aspect of the invention may be provided together.

In accordance with the multi-component foodstuff of the first aspect of the invention, one composition may comprise, for example at least 40% fat (on a fat:energy ratio) and a different composition may comprise, for example, at least 40% protein on a protein:energy ratio. The sources of fat, protein and carbohydrate can be provided, for

example by two or more different dry kibbles, for example two or more of the kibbles as follows:-

	PER:FER:CER	PME (kcal/100g)
Carbohydrate enriched	26%/22%/52%	344
Protein enriched	51%/23%/26%	336
Fat enriched	27%/45%/28%	404

wherein PER = protein:total energy ratio
FER = fat:total energy ratio
CER = carbohydrate:total energy ratio
PME = predicted metabolisable energy.

A second aspect of the present invention provides a multi-component foodstuff according to the first aspect in the invention, for use in providing an optimum macronutrient diet for an individual animal. Such a selection can be represented by the triangle of Figure 1 (representing dry diets of varying macronutrient profile). The foodstuff according to the first aspect of the invention allows the animal to regulate the total intake of each macronutrient. It allows the animal to regulate on fat intake in carbohydrate free diets. It allows the animal to regulate on carbohydrate intake in carbohydrate-containing diets. All of these have been shown to be desired in animals.

The experimental work showed a preferred protein, fat and carbohydrate intake (a target). The effects are large enough to affect the total daily intake at the expense of caloric intake. Further, the effects are large enough to affect product selection in a choice situation.

All preferred features of the first aspect, also apply to the second.

A third aspect of the invention provides a foodstuff according to the first aspect of the invention, for use in animal weight maintenance. The present invention provides a multi-component foodstuff which allows animals to self-regulate their food intake. Where the animal can self-select to achieve a target macronutrient content, the total consumed is optimal, thus contributing to weight maintenance. In this text, weight maintenance includes a contribution to the prevention or reduction of obesity.

All preferred features of the first and second aspects, also apply to the third aspect.

A fourth aspect of the present invention provides a foodstuff according to the first aspect of the invention, for use to promote animal health benefit.

Such health benefits include improved immune function, reduced oxidative damage and DNA damage, ability to cope with oxidative stress/challenge, improved life expectancy, improved metabolic rate and function, improved gut function and digestibility, reproductive efficiency, improved behaviour, cognitive function and improved disease resistance.

A fifth aspect of the invention provides a foodstuff according to any one of the first to fourth aspect of the invention, wherein the compositions are separately packaged. The compositions may be separately packaged or packaged together.

A sixth aspect of the invention provides food compositions, for use in a foodstuff according to the first to fifth aspect of the invention.

A seventh aspect of the invention provides a method of providing optimum macronutrient diet to an individual animal, the method comprising feeding said animal a multi-component foodstuff according to the first aspect of the invention. The different food compositions of the foodstuff are provided simultaneously.

An eighth aspect of the invention provides a method of weight maintenance, the method comprising feeding said animal a multi-component foodstuff according to the first aspect of the invention. The multi-component foodstuff may be provided in unlimited quantities. The different food compositions of the foodstuff are provided simultaneously. By weight maintenance is meant maintaining a desired weight range or weight loss towards a desired weight.

A ninth aspect of the invention provides a method of promoting animal health benefit, the method comprising feeding said animal a multi-component foodstuff according to the first aspect of the invention. The foodstuff may be provided in unlimited quantities. The different food compositions of the foodstuff are provided simultaneously.

All preferred features of the first aspect of the invention, also apply to the other aspects of the invention, *mutatis mutandis*.

In aspects of the invention which describe "feeding", it is meant allowing the animal access to the foodstuff of the invention to feed from.

The present invention is based on the observation that when consuming food, animals are attempting to reach a target intake of each of the three macronutrients (protein, carbohydrate and fat) within a given time period. The invention describes a multi-component foodstuff which allows an individual animal to reach its target consumption of protein, fat and carbohydrate. According to the invention, the animal is offered a choice of two or more food compositions of differing macronutrient ratios. The foods can be offered continuously or at every meal occasion. The target macronutrient consumption will vary over time depending on factors such as life stage, sex, sexual activity, illness, seasonal variation, environment, stress levels etc. Therefore continuous access to these food components allows the animal to vary its consumption of each macronutrient independently and to a level optimal for any point

in time. Self-selection also occurs within individual meals. The invention offers benefits of increased enjoyment of eating and health benefits for the animal.

Existing food products are formulated to provide a specific fixed ratio of carbohydrate, fat and protein. This invention allows the cat to vary its consumption of each of the macronutrients independently and to a level optimum for any point in time.

The present invention provides advantages. It offers an optimum diet for an individual animal based on that animal's metabolic needs as opposed to transitory sensory preferences.

The invention provides a solution to the problem of providing palatable foods for animals, as well as offering benefits to the animal of increased acceptance/increased enjoyment in feeding. Furthermore the invention provides an increased enjoyment/satisfaction by the carer/owner of a pet (companion) animal.

The enjoyment of the animal and/or increase in acceptance/palatability can be determined, for example, by one or more of the following:-

- an increase in the quantity of foods consumes;
- a decrease in the frequency of refusals to eat over an extended period of time;
- an increase in enthusiasm during the meal as indicated by a reduction in the time taken to start a meal and/or an increase in the speed at which food is consumed;
- the animal chooses the food over another food;
- the animal refuses other foods;

or by any other behaviour by a pet animal which is taken by the owner/carer to be an indication of enjoyment of the food, for example:-

- the animal rubs around the owner/carer when serving the food;
- the animal is inactive/rests or sleeps after eating;
- the animal licks itself or washes after eating.

In addition to these benefits, providing a food which matches the optimum-macronutrient ratio for a particular animal offers health benefits to the animal, such as maintenance of a healthy weight body mass index, obesity prevention, improved immune function, reduced oxidative damage, and DNA damage, ability to cope with oxidative stress challenge, improved life expectancy, improved metabolic rate and function, improved gut function and digestibility, reproductive efficiency, improved behaviour, cognitive function and improved disease resistance.

The present invention is now described with reference to the following non-limiting examples:-

<u>Examples</u>

<u>Example 1</u>

<u>Study to assess the effect of dietary macronutrient profile on food selection in cats</u>

<u>Summary</u>

- Adult cats were fed homogenised diets composed of soy isolate, chicken breast, lard and either carob solution or water. The diets were isocaloric and consisted of a range of ratios of protein to fat energy (P-F:ER), these being 10% PER/90% FER (a PER thought to be close to the cat's minimum protein requirement), 40%

PER/60% FER (a PER typical for a canned product) and 70% PER/30% FER. Three flavours of different relative preference were included with the diets, such that each of the 3 groups of cats received different flavour-diet combinations.

- During the initial 7 days of self-selection/3-way preference, the naïve cats (with no prior experience of the diets and flavours) appeared to make their diet selection based on the hedonic cues associated with the diets. They selected the diet associated with the preferred flavour, irrespective of the amount of soy isolate and lard.

- During the 39 days of learning/training, the cats changed their macronutrient selection and, in terms of mean food intake, responded differently to the macronutrient profile and flavour of the diets.

- During the final 7 days of self-selection/3-way preference, these now experienced cats showed different feeding responses to the initial self-selection and appeared to select diets on some other basis, consequently selecting a different macronutrient profile overall. It appeared that the experienced cats had 'learned' about the macronutrient profile of the diet and changed their preferences accordingly, with the low protein/high fat food consistently rejected, regardless of the flavour added. The protein to fat ratio below which the product is rejected is not known and will be investigated in further studies. This should identify the minimum protein level for cat food, based on acceptance rather than nutritional requirement.

- Investigation of protein to fat energy ratio (P-F:ER) intake as a potential driver of diet selection showed that the mean P-F:ER consumed during the initial self-selection phase was influenced by the hedonic cues, with each test group having a mean PER intake driven by the diet with which the preferred flavour was associated (mean PER intake 34%; FER intake 66%). In contrast, the mean P-F:ER

intake of the same cats once they were experienced was more consistent, with less influence from the hedonic cues during the final self-selection (mean PER intake 49.8%; FER intake 50.2%).

- Overall these results suggest that repeated exposure to these experimental diets over an extended period of feeding led to cats changing their dietary preferences, in order to select specific macronutrient profiles. In doing so, they responded less to the hedonic cues and more to the underlying nutritional cues.

1 Introduction

The traditional approach to palatability has been that taste, smell and texture are very important drivers of intake on initial exposure to a food. More recent studies in cats have led to the hypothesis that, with experience, the underlying nutrition may override these sensory cues, thus causing food choice to change (if it is of benefit for the animal to do so).

The results of previous work suggest that repeated exposure to experimental chicken and lard-based diets over an extended period of feeding leads to cats changing their dietary preferences, in order to select specific macronutrient profiles. In doing so, they respond less to the hedonic cues and more to the underlying nutritional cues. This observation opposes the established belief that cats do not possess 'nutritional wisdom', since hedonics are the only driving factor in food selection.

The aim of this study was to determine whether cats 'learned' about the macronutrient profile of the diet, such that the initial hedonic response was subsequently influenced by physiological responses (which may vary with the macronutrient profile of the diet). Cats were tested prior to experiencing the experimental diets and flavours, then after a period of monadic, repeated exposures to the diets, to determine if their feeding responses had changed through experience.

In order to control the macronutrient profile of the diet within more defined limits than are achievable using a typical wet product recipe, relatively 'clean' sources of macronutrients were used. In the second part of this first phase of work, the macronutrients protein and fat were investigated. - soy isolate was used as the predominant protein source, with some chicken breast present in all diets, and lard was used as a fat source.

Diets were designed consisting of increasing levels of protein (soy isolate and chicken), combined with decreasing levels of fat (lard). In order to 'confuse' the cats and mask the natural smell and flavour of the diets, additional flavour cues were added (see 'Methods' section), to reduce selection of a product purely on the basis of its inherent smell or flavour. This should demonstrate if the cats 'learned' about and selected diets on the basis of their protein or fat content *per se,* when fed over an extended period. The trial was also designed to indicate whether cats preferred a diet containing a specific level of protein and/or fat, when offered an *ad libitum* choice of the 3 diets.

2 Methods

2.1 Animals

Cats (n=27) were selected that had no prior experience of the experimental diets or flavours. The cats were housed individually and were socialised as a group every day.

Cats were randomised into balanced groups by age, sex and bodyweight.

2.2 Diets

Three isocaloric diets were fed, all designed to supply 70 kcal ME (metabolisable

energy) per 100g final product. The diets consisted of a range of ratios of protein to f energy (P-F:ER), these being 10% PER/90% FER (a PER thought to be close to tl cat's minimum protein requirement), 40% PER/60% FER (a PER typical for a cann product) and 70% PER/30% FER. In this study, the diets were essential carbohydrate-free, with the calorie deficit remaining after inclusion of prote provided by fat calories.

Diets were prepared daily and consisted of a homogenised mixture of powdered sc isolate, cooked chicken breast, lard and either carob solution (3% or 0.5%, w/w) water. The diets contained differing amounts of carob solution, in order to accomplis similar consistencies. In a preliminary study diets with the same base recipe wer prepared with 3 different concentrations of carob solution (0.75%, 1.5% and 3%, w/w and were fed in a repeated intake test to a panel of 24 cats. The results indicated th the concentration of carob did not affect the relative acceptance of the diets, with n significant difference between the mean intakes recorded ($p = 0.66$).

These recipes were formulated predictively using a combination of proximate analyse of powdered soy isolate (from ICN), processed chicken breast and lard, and publishe food composition data, to give final products with PER's of 10%, 40% and 70% and a energy density of 70 kcal/100g final product. Textural differences between the diet were reduced by homogenising the diets, and including varying amounts of carol solution or water, thus making the diets relatively similar in consistency.

The nutritional content of each diet was estimated using the above sources o information. Diets were formulated to meet minimum WALTHAM Cat Nutrien Guidelines for adult maintenance, through addition of vitamin and mineral mixes taurine and L-methionine (soy isolate being low in sulphur-containing amino acids and were made up fresh on a daily basis.

Three flavour systems of different relative preference were included with the diets

such that each group of cats received different flavour-diet combinations. This made a total of nine test diets (tables), all of which were fed. The concentration of each flavour was determined by mixing it with the diet and tasting the different flavour-diet combinations. The flavours were added at concentrations which were just detectable by humans by aroma and taste, so were presumed to be detectable by cats. The inclusion level of the flavours was the same for each diet, irrespective of the protein content. The three flavours used were Quest rabbit (0.06% (w/w); 27 drops rabbit flavour added per kg product), Firmenich fish powder (1.5% (w/w); 15g fish powder added per kg product) and Firmenich orange oil (0.03% (w/w) of a 19% (w/w) solution of orange oil in sunflower oil; 13 drops diluted orange oil added per kg product). [Diluted orange oil was prepared as 10 drops orange oil in 1g sunflower oil].

The cats were split into 3 groups (n=9 per group), such that each group of cats received different flavour-diet combinations throughout the study, as shown in Table 2.

Table 1: Flavour-diet combinations fed.

Product	PER	Flavour
A	10%	fish (F1)
B	40%	rabbit (F2)
C	70%	orange (F3)
D	10%	orange (F3)
E	40%	fish (F1)
F	70%	rabbit (F2)
G	10%	rabbit (F2)
H	40%	orange (F3)
I	70%	fish (F1)

Table 2: Flavour-diet matrix fed.

Cats	Diet-flavour combinations		
Group 1	10% + fish	40% + rabbit	70% + orange
Group 2	10% + orange	40% + fish	70% + rabbit
Group 3	10% + rabbit	40% + orange	70% + fish

2.3 Feeding protocols

The feeding protocol consisted of an acclimatisation pre-feed followed by 4 different feeding regimens – an initial self-selection/3-way preference phase, a learning/training phase, a final self-selection/3-way preference phase and a challenge to investigate preferred flavour selection.

- *Acclimatisation pre-feed (7d)*
  - To familiarise the cats to the format of the homogenised diets (without exposing them to the soy/chicken/lard diets), a pre-feed of Feline Concentration Instant diet (FCID) was included.
  - Daily requirements of FCID were calculated on the basis of individual cat bodyweights, then an additional 25% was added, so that each cat (in principle) was being fed to appetite.
  - Cats were offered a third of their daily requirement of FCID in the morning, and the remainder of their requirement in the afternoon, which remained overnight.
  - The amount of food offered was increased to 50% above requirement for most of the cats, who consistently consumed all food offered. Food offered was not

increased beyond this, since those cats which continued to consume all of this increased ration were deemed to be overeating.

- All food intakes were recorded manually every time food was replaced.

- *Naïve self-selection/3-way preference phase (7d)*
  - For the subsequent 7 days, each cat was given *ad libitum* access to all 3 of the experimental diets.
  - Food was replaced with fresh twice per day - 150g of each diet was offered in the morning, which was replaced with 250g fresh food in the afternoon, which remained overnight.
  - The position of the products was rotated daily.
  - Food intakes and meal patterning were recorded constantly. In addition, all food intakes were recorded manually every time food was replaced.

- *Learning/training phase (39d)*
  - During the learning/training phase, each cat should have received a single product each day, with the 3 diets fed in daily rotation for 30 days. Each cat should therefore have experienced each test diet 10 times.
  - After completion of cycle 8, supplies of soy isolate ran out, so all cats were transferred to unflavoured FCID for 12 days. The learning phase re-started and the cats were given 5 further cycles of learning (cycles 13 to 18). In total, the cats experienced each test diet 13 times.
  - In order to reduce sequence effects, e.g. to avoid a cat always receiving diet B after diet A, 3 different orders of diet presentation were followed.
  - Each cat received 200g of food in the morning, which was replaced with 300g of the same diet in the afternoon, which was left overnight.
  - Food intakes and meal patterning were recorded constantly. In addition, all food intakes were recorded manually every time food was replaced.

- *Experienced self-selection/3-way preference phase (7d)*
  - For the subsequent 7 days, each cat was given ***ad libitum*** access to all 3 of the experimental diets they had experienced in the learning phase.
  - Food was replaced with fresh twice per day - 150g of each diet was offered in the morning, which was replaced with 250g fresh food in the afternoon, which remained overnight.
  - The position of the products was rotated daily.
  - Food intakes and meal patterning were recorded constantly. In addition, all food intakes were recorded manually every time food was replaced.

NOTE: The amount of food offered was increased in cats that consistently consumed all of the food provided.

2.4 Bodyweights

Bodyweights were recorded twice per week and closely monitored to assess adequate food intakes.

2.5 Data analysis

During the learning phase, graphs are expressed with 'Cycle' on the x-axis, each 'Cycle' being a pseudo-randomised 3-day rotation of products, and therefore an exposure to each of the diets.

3 Results

3.1 Diets

3.1.1 Nutritional properties

As mentioned previously, the diets were formulated predictively to give final products with PER's of 10%, 40% and 70% and an energy density of 70 kcal/100g final product.

Proximate analysis values of the diet ingredients were used, together with Atwater factors (protein 4 kcal/g, fat 9 kcal/g, carbohydrate 4 kcal/g), to calculate the maximum PME of each ingredient.

The maximum PME for each of the diet ingredients was then used to calculate the PME for each of the diets, based on the proportions of the ingredients in each formulation. Corrected PER was calculated using these corrected ME values and the protein values from the diet analysis. The protein content (determined from analytical results) was multiplied by 4 kcal/g and expressed as a percentage of the ME of the diet. Corrected FER was calculated by difference, the diets consisting only of protein and fat calories.

3.1.2 Flavours

The relative preference for all of the flavour-diet combinations plus the unflavoured diets was assessed.

- Statistical analysis of the data by multifactor ANOVA showed that there was a significant difference between the mean intakes of the test diets ($p < 0.001$).
- The results confirmed that the relative preference for the three flavour systems used was fish > rabbit = orange oil ($p < 0.001$).

- The results also showed that the three flavours maintained this ranking across all three base diets (10%, 40%, 70% PER) to which they were added.
- It was interesting to note that for the unflavoured diets, the relative preference was 40% > 70% > 10%, however, these differences were not statistically significant. The 40% PER diet was generally preferred over the other test diets, even when flavours were associated with them.

3.2 Animals

Four cats were removed from the study after 2-3 weeks on the trial products. This was due to consistently low food intakes.

3.3 Testing responses to novel flavours and macronutrient profiles: Naïve self-selection phase

During the self-selection phase, all 3 test diets were fed every day, with each cat having *ad libitum* access to sample from the 3 diets throughout the day.

During this initial phase, the cats had no prior experience of these flavours and experimental diets

- In each test group, cats ate a greater proportion of the diet combined with fish flavour, fish being the preferred flavour hedonically.

- These data suggest that cats naïve to the diet format and flavours appeared to make their diet selection based on the hedonic cues associated with the diet. They selected the diet associated with the preferred flavour, irrespective of the amount of soy isolate and lard.

- Combining the data from all 3 test groups and assessing diet selection based on protein alone, during the naïve self-selection phase, there was a significantly greater proportion of the 10% PER diet eaten compared with the 40% and 70% PER diets ($p<0.01$).

- For all cats, determination of diet selection based on flavour alone showed that the preference for fish flavour over rabbit and orange flavours was clearly apparent ($p<0.001$).
- Looking at individual cats, the majority of cats within each test group showed similar diet preferences.
- There was no common feature in those cats which showed a different diet selection to the rest of each test group.

3.4 Training cats to recognise links between flavours and macronutrient profiles: Learning phase

During the learning/training phase, each diet was fed on a different day, i.e. only one diet was fed per day. Food intake data were analysed by a 3-day cycle, during which each cat experienced each of the 3 diets within its test group.

Graphical comparison of the mean proportions of food eaten in all the cats at the start (cycle 1) and end (cycle 17) of the learning phase by either flavour alone or PER alone showed interesting differences.

- There was a significant difference ($p<0.001$) between the mean intakes of fish, rabbit and orange flavour at the start of the learning phase, with fish flavour preferred over the other 2 flavours. However, by the end of the learning phase, there was no significant difference between the proportions of the flavours consumed.
-

- In contrast, there was no significant difference between the mean intakes of 10%, 40% and 70% PER at the start of the learning phase, however, by the end of the learning phase, the mean intakes of all of the diets were significantly different, with 70% > 40% > 10% PER.

- As seen previously, the intake of the 10% PER diet during the learning phase was markedly lower than the total intake of the other diets, particularly at the end of the learning phase.

- These data suggest that in terms of mean food intake, macronutrient profile and flavour behaved differently during the learning phase. The macronutrient profile selected changed over the 39 days of feeding (excluding the days during which FCID was fed).

3.5 Testing trained links between flavours and macronutrient profiles: Experienced self-selection phase

During the self-selection phase, all 3 test diets were fed every day, with each cat having *ad libitum* access to sample from the 3 diets throughout the day.

Between the first 'naïve' self-selection phase and this second self-selection phase, the cats experienced a training period to help them associate certain flavours with specific macronutrient profiles, and were thus classified as 'experienced'.

- Diet selection in the experienced self-selection differed to that seen in the naive self-selection, where fish flavour (irrespective of PER) was preferred in each test group.
- In experienced group 1 cats, cats ate a significantly greater proportion of 70% PER + orange compared with 10% PER + fish and 40% PER + rabbit.

- In experienced group 2 cats, cats ate significantly different proportions of each diet, with 40% PER + fish > 70% PER + rabbit > 10% PER + orange.
- In experienced group 3 cats, cats ate significantly different proportions of each diet, with 70% PER + fish > 40% PER + orange > 10% PER + rabbit.
- In all test groups, the 10% PER diet was rejected relative to the other two test diets. In group 1, this rejection was not as great as in the other test groups. One hypothesis for this may be that in group 1, the 10% PER diet was combined with fish flavour, thus improving the hedonic acceptability of the product.

- These data suggest that cats with experience of the diet format and flavours consistently rejected the low protein/high fat diet regardless of the flavour associated with it. Experienced cats appeared to base their diet selection on some other attribute of the products, rather than purely on hedonics.

- Combining the data from all 3 test groups and assessing diet selection based on protein alone, during the experienced self-selection phase, there were significantly different proportions of each diet consumed, with 70% > 40% > 10% PER ($p<0.001$) (Figure 10). This was different to the diet selection seen in the same group of cats during the naïve self-selection phase, where 10% > 40% = 70% PER.

- For all cats, determination of diet selection based on flavour alone showed that the preference for each flavour was significantly different, with fish > orange > rabbit ($p<0.001$). This was similar to that seen in the naive self-selection phase.

<u>*3.6 Investigation of daily diet selection within each test group*</u>

Please note that all food intakes in this section are based on manually recorded data, without any correction for evaporative losses from the food. Typical evaporative loss from these homogenised products has been estimated as 6-7% of the initial weight of

the food over a 16 hour period (i.e. overnight).

The previous sections (3.3, 3.4 and 3.5) described mean food intakes within the test groups, however, it is of interest to compare the daily food intakes in more detail.

3.6.1 Group 1

Figure 2 shows the daily diet selection of group 1 cats throughout the study (naïve self-selection, learning and experienced self-selection phases).

- Naïve self-selection: 10% PER + fish was markedly preferred over the other two test diets from day 1 of the naïve self-selection. Intakes of 40% PER + rabbit and 70% PER + orange were similar.
- Learning phase: In cycle 1, intakes of all 3 test diets were very similar, but during the learning phase, the proportion of 10% PER + fish consumed decreased and the proportion of 40% PER + rabbit consumed increased. After the period of FCID, intakes of the 40% and 70% PER diets separated, with 70% PER + orange preferred over 40% PER + rabbit.
- Experienced self-selection: the proportion of 70% PER + orange consumed was greater than the other two diets from day 1. Slightly more 40% PER + rabbit was consumed than 10% PER + fish; the proportion of 10% PER + fish consumed was approximately 20%.

<u>*3.7 P-F:ER selection as a potential driver of macronutrient selection*</u>

The mean PER intake was calculated for each phase of the trial from food intake data:

Mean PER eaten per day = (Amount test diet eaten (g) x PER of test diet) / Total amount eaten (in g)*

* For self-selection phase (naïve/experienced) = sum of 3 test diets eaten per day (in g)

For learning phase = sum of test diets eaten over 3-day cycle (in g)

Self-selection phase (naïve/experienced): Mean PER intake per day = Average of PER eaten per day

Learning phase: Mean PER intake per cycle = Average of PER eaten across 3-day cycle

The mean FER intake was calculated by difference from the above data.

Throughout the naïve self-selection phase, the mean P-F:ER intake per cycle was calculated (Table 10). The PER intake varied between each test group of cats, the variation driven predominantly by the PER of the diet with which fish flavour was associated (since in each test group, naïve cats consumed most of the diet paired with fish flavour – see section 3.3). It should be noted that if diet selection was completely random (i.e. sampling from 10%, 40% and 70% PER diets), the mean PER intake would be 40% (with an FER intake of 60%).

All 3 diets were offered together in a sufficient quantity for the cats to achieve their daily energy requirement by eating only one of the products if they desired.

Throughout the learning phase, the mean P-F:ER intake per cycle was calculated. The PER intake was higher than in the previous phase and was fairly constant for each group of cats. The mean PER intake gradually rose throughout the learning phase. On average, the PER selected reflected random sampling from the diets, as described above, i.e. mean PER intake of 40% and FER intake of 60%. This was seen clearly in groups 1 and 2, where the mean PER intake was approximately 40% and FER intake 60%, suggesting that the cats were consuming equal amounts of each diet. When the amount of each diet eaten (in grammes) was compared, this was not the case and

different quantities of each diet were being consumed. These data suggest that the mean PER and FER intakes were not derived from random diet sampling.

Throughout the experienced self-selection phase, the mean P-F:ER intake per cycle was calculated. Compared with the naïve self-selection, the response during the experienced self-selection was very different. The mean PER intake remained remarkably constant within each group of cats and was at a higher level than seen initially.

4 Conclusions

During the initial 7 days of self-selection/3-way preference, the naïve cats (with no prior experience of the diets and flavours) appeared to make their diet selection based on the hedonic cues associated with the diets. They selected the diet associated with the preferred flavour, irrespective of the amount of soy isolate and lard.

During the 39 days of learning/training, the cats changed their macronutrient selection and, in terms of mean food intake, responded differently to the macronutrient profile and flavour of the diets.

During the final 7 days of self-selection/3-way preference, these now experienced cats showed different feeding responses to the initial self-selection and appeared to select diets on some other basis, consequently selecting a different macronutrient profile overall. It appeared that the experienced cats had 'learned' about the macronutrient profile of the diet and changed their preferences accordingly, with the low protein/high fat food consistently rejected, regardless of the flavour added. The protein to fat ratio below which the product is rejected is not known and will be investigated in further studies. This should identify the minimum protein level for cat food, based on acceptance rather than nutritional requirement.

This study confirms that the macronutrient profile (in this case, protein and fat) can affect the long term feeding performance of a food. Feeding regimen is important in this, since the cats required a 'learning' period of repeated exposures before changing their diet selection.

Investigation of mean P-F:ER intake as a potential driver of feeding behaviour showed that naïve cats consumed a variable P-F:ER during the self-selection phase, which was driven by hedonic cues (mean PER intake 34%; FER intake 66%), whereas the mean P-F:ER intake of the same cats once they were experienced tended to be more constant, and overall gave a higher PER value during the self-selection phase (mean PER intake 49.8%; FER intake 50.2%). These results are similar to those seen previously, where the mean PER intake of naïve cats was 42.5% and experienced cats was 54.9%. It appears that P-F:ER intake is a driver of feeding behaviour and macronutrient selection in the long term.

A graph showing details of the preferences is shown in Fig 2.

The results show that cats which are naive to the diets and flavours select diets based on hedonic cues (i.e. added flavours), whereas experienced cats appear not to use the hedonic cues and select a different macronutrient profile overall. Macronutrient profile therefore affects the long-term feeding performance of a food and diet selection. The study also shows that animals will reject a food if its protein level is too low – the 10% PER/90 FER diet was consistently rejected after a period of learning, irrespective of the flavour added.

The study shows that animals try to make food selections in order to achieve a metabolically desirable mean PER intake.

Example 2

Effect of macronutrient profile on the long-term acceptance of food

Summary

This trial aimed to establish whether the naïve response to the hedonic properties of three diets (carbohydrate enriched, protein enriched and fat enriched) could be modified by a monadic learning phase.

12 adult cats were fed 3 diets during this study following the feeding protocol: 7-days of self-selection/ 3-way preference followed by 24-days of monadic learning (one product per day) and finally 7 days of self-selection/3 way preference. Cats had approximately 22 hours access to food every day during the trial.

10 out of the 12 cats completed the study; 2 cats were removed from the study due to inadequate food intakes. The mean percentage change in bodyweight during the trial for the cats that completed the study was +2.39%

The naïve response of the cats to the 3 diets was that the protein enriched diet had the highest mean intake (22g) whilst the carbohydrate and fat enriched diets were slightly lower (mean intake 16g and 17g respectively).

The experienced response of the cats to the 3 diets was that the carbohydrate enriched diet was almost completely rejected, with a very low mean intake (6g). There was a slight increase in the mean intake of the fat enriched diet (21g), compared to the naïve response, and a large increase in mean intake of the protein enriched diet (41g).

Investigation of the proportion of energy intakes of protein, fat and carbohydrate (P/F/CER) showed naïve cats consumed 36%/30%/34% respectively, averaged over

all cats and all days of the naïve self-selection phase. The same analysis of P/F/CER during the experienced self-selection showed the proportion of macronutrients consumed was 42%/30%/29% respectively averaged over all cats and all days of the experienced self-selection phase

In summary, the carbohydrate enriched diet was almost completely rejected after a monadic learning phase whilst selection of the protein enriched diet almost doubled in terms of g intake. Analysis of the macronutrient selection of the cats showed a 6% increase in protein and 5% decrease in carbohydrate intake after a period of monadic learning, the proportion of fat intake remained constant

Introduction

A previous study was run to establish whether the naïve response to the macronutrient profile of three dry diets could be modified by a period of monadic learning on a panel of 163 cats in domestic homes in Germany.

The aim of this study was to use the same diets and trial design on a further panel of cats.

Methods

Animals

Cats (n = 12) were selected that had been fed dry kibble diets throughout life, from the time they were weaned.
The cats were housed individually and were socialised as a group every day.

Diets

Three dry kibble diets were fed during the study. One enriched with protein, one enriched with fat and one enriched with carbohydrate. Analysis of the diets provided the predicted metabolisable energy (PME) content of each diet, the values of which ar shown in Table 1. The protein, carbohydrate and fat content of each diet was analysec and calculated to provide the ratio of each macronutrient relative to the total energy (PME) of each diet i.e. Protein/Fat/Carbohydrate Energy Ratio (P/F/CER in Table 1).

Table 1: PME and macronutrient energy ratios for the diets used

Diet Code	**PER:FER:CER**	**PME (Kcal/100g)**
A (carbohydrate enriched)	26%/22%/52%	344
B (protein enriched)	51%/23%/26%	336
C (fat enriched)	27%/45%/28%	404

Feeding Protocols

The feeding protocol consisted of 3 different feeding regimes – an initial self-selection/3 way preference, a learning/training phase and a final self selection/3-way preference phase.

Naïve self selection/3-way preference phase (7d)

- Each cat received ad libitum access to all 3 experimental diets.

- 150g of each diet was offered at 10:15am and left available until 8:15am the following morning giving each cat 22 hours exposure to the diets on each day. This feeding cycle was repeated daily for 7 days.
- The position of the diets was rotated daily.
- Food intakes and feeding patterns were recording constantly. In addition, food intakes were recorded manually every time food was replaced.

Learning/training phase (24d)

- During the learning/training phase, each cat received a single test diet each day
- The three diets experienced by the cats during the naïve self selection were fed in daily rotation for the 24 days. Each cat therefore experienced each experimental diet 8 times.
- 150g of a single diet was offered at 10:15am and left available until 8:15am the following morning. This feeding cycle was repeated daily for 24 days, giving each cat 22 hours exposure to the diet on each day.
- Cats were randomly assigned to one of 6 groups with each group receiving the diets in a different rotation sequence.
- Food intakes and feeding patterns were recorded constantly. In addition, food intakes were recorded manually every time food was replaced.

Experienced self-selection/3-way preference (7d)

- Each cat received ad libitum access to all 3 experimental diets.
- 150g of each diet was offered at 10:15am and left available until 8:15am the following morning giving each cat 22 hours exposure to the diets on each day. This feeding cycle was repeated daily for 7 days.
- The position of the diets was rotated daily.

- Food intakes and feeding patterns were recording constantly. In addition, food intakes were recorded manually every time food was replaced.

Bodyweights

Bodyweights were recorded twice weekly and closely monitored to assess adequate food intakes.

Data Analysis

Feeding pattern data were analysed by computer software that splits the data into individual meals, giving time, duration, rate and latency of each. These could then be analysed for each cat and each diet.

Note: During the learning phase, graphs are expressed with 'cycle' on the x-axis. Each 'cycle' is a pseudo-randomised 3-day rotation of products, and therefore includes an exposure to each of the 3 diets.

Results

Animals

Two cats were removed from the trial due to consistently poor food intakes. All food intakes for these cats are excluded from this report thus decreasing the sample size to 10 cats.

The mean percentage change in bodyweight, averaged over all cats that completed the study from the start of the trial to the end of the trial was +2.4%. The progress of this change is shown in figure 3.

There were only 2 cats that completed the study which showed a decrease in bodyweight (-2.53% and -0.79% respectively). 2 cats' bodyweights increased more than 6% during the trial. All other cats showed a, less than 5%, increase in bodyweight. 6 out of 10 of the cats showed a dip in bodyweight during the first couple of weeks of the trial this is often seen in cats that change diets.

Naïve self-selection phase

During the naïve self-selection phase, all 3 test diets were fed every day. All cats received *ad libitum* access to the diets for 22 hours each day. The diets were removed at 8:15am every morning and replaced with fresh diet at 10:15am.

All cats were naïve to the experimental diets except for 3 cats who were exposed to each diet twice during a six-day period.

Figure 4 shows the daily mean food intake, averaged over all cats, for each diet during this 7-day phase. Mean daily intakes of each diet were similar, intakes of diet B (high protein) were slightly higher on day 2,4,5 and 6 than the other 2 diets.

Figure 5 shows the mean food intake, averaged over all cats and all days for each diet during the 7-day naïve self-selection phase. On average, the intake for diet B (high protein) was slightly higher than both diet A and diet C ($p = 0.02$)

Figure 6 shows the proportion of total eaten of each diet, averaged over all days, for each cat during naïve self-selection

Learning Phase

Figure 7 shows the daily mean intake, averaged over all cats, for each diet during each of the eight 3-day cycles. In the figure, significantly different $p<0.001$.

Figure 8 shows the daily mean intake, averaged over cats and all cycles, for each diet during the learning phase (including results from naïve self-selection).

Experienced self-selection

Figure 9 shows the daily mean intake, averaged over all cats, for each diet during experienced self-selection. In the figure, significantly different $p<0.001$.

Figure 10 shows the mean daily intake, averaged over all cats and all days, for each diet during experienced self-selection.

Figure 11 shows the mean daily intake, averaged over all cats and all days, for each diet during experienced self-selection.

<u>P/F/CER selection as a potential driver of macronutrient selection</u>

Each cats mean energy ratio intake was calculated for each phase of the trial from the food intake data.

The calculation used was:

Mean P/F or CER eaten per day =

$$\text{Mean PER eaten per day} = \frac{\sum_{\text{Over all 3 test diets}} (\text{Amount of test diet eaten (g)} \times \text{PER of test diet})}{\text{Total amount eaten (g)}\,*}$$

*

For self-selection phase (naïve/experienced) = sum of 3 test diets eaten per day (g)

For learning phase = sum of 3 test diets eaten over 3-day cycle (g)

The daily/per cycle mean PER intake was calculated for each cat.

This was repeated for both FER and CER.

- Table 3 shows the mean daily/per cycle PER, FER, CER for each phase of the trial, averaged over all cats.

Table 3: Mean cycle PER, FER and CER intake for each phase

	Naïve self-selection	Learning	Experienced self-selection
PER	36.2	36.2	41.6
FER	29.6	31.7	29.7
CER	34.2	32.1	28.8

Table 4: Mean intake (g) of each diet during each phase

	Diet A (High Carbohydrate)	Diet B (High Protein)	Diet C (High Fat)
Naïve self-selection	15.8	21.6	17
Learning	35.7	68.4	70.2
Experienced self-selection	5.5	40.9	20.5

Figure 12 shows the mean daily PIF/CER intake during the trial.

Example 3

Effect of macronutrient profile on the long-term acceptance of food: *Effect of varying Protein and Fat Energy Ratios*

Summary

12 adult cats were fed 3 diets (one high in protein, one high in fat and one intermediate) during this study following the feeding protocol: 7-days of self-selection/ 3-way preference followed by 24-days of monadic learning (one product per day) and finally 7 days of self-selection/3 way preference. Cats had approximately 22 hours access to food every day during the trial.

All 12 cats completed the study. The mean percentage change in bodyweight during the trial for the cats was +2.2%

The naïve response of the cats to the 3 diets was that the high protein and intermediate diets were preferred (mean intakes 19g and 22g respectively) over the high fat diet (mean intake 11g)

The experienced response of the cats to the 3 diets was similar to that seen during the naïve self-selection in that the high protein and intermediate diets were preferred (mean intakes 26g and 25g respectively) over the high fat diet (mean intake 6.4g).

Investigation of the proportion of energy intakes of protein and fat (P/FER) showed naïve cats consumed 37%/38% respectively, averaged over all cats and all days of the naïve self-selection phase. The same analysis of P/FER during the experienced self-selection showed the proportion of macronutrients consumed was 39%/36%respectively averaged over all cats and all days of the experienced self-selection phase.

Cats consumed equal amounts of each diet during the monadic learning phase. Analysis of feeding pattern on the days cats were offered the high fat diet alone suggests the cats were either "holding out" for something better and then eating the diet, or, were regulating the speed of fat intake.

In summary, the high protein and intermediate diets appeared to be hedonically more palatable than the high fat diet. The increased rejection of the high fat diet alongside the increase in PER and decrease in FER during the experienced self-selection (+2% and –2% respectively) provides evidence that the macronutrient profile of the high fat/low protein (22%PER/53%FER) diet is less preferable than the intermediate (34%PER/42%FER) and high protein (48%PER/26%FER) diets.

Introduction

This study is to establish whether the naïve response to the macronutrient profile of dry diets can be modified by a period of monadic learning. The aim of this study was to assess diets with equal carbohydrate energy ratios (CER) but variable protein and fat energy ratios such that one diet had a high fat energy ratio (FER), another had a high protein energy ratio (PER) and a third diet had an intermediate energy ratio of protein and fat.

Methods

Animals

Cats (n = 12) were selected that had been fed dry kibble diets throughout life, from the time they were weaned.

The cats were housed individually and were socialised as a group every day.

Diets

Three dry kibble diets were fed during the study. The diets were designed to all contain the same level of carbohydrate whilst one diet was enriched with protein, one enriched with fat and the other was the intermediate of the other two diets. Analysis of the diets provided the predicted metabolisable energy (PME) content of each diet, the values of which are shown in Table 1. The protein, carbohydrate and fat content of each diet was analysed and calculated to provide the ratio of each macronutrient relative to the total energy (PME) of each diet i.e. Protein/Fat/Carbohydrate Energy Ratio (P/F/CER in Table 1).

Table 1: PME and macronutrient energy ratios for diets

Diet Code	PER:FER:CER	PME (Kcal/100g)
B (High Protein)	48%/26%/26%	346
C (High Fat)	22%/53%/25%	391
F (Intermediate)	34%/42%/24%	427

Feeding Protocols

The feeding protocol consisted of 3 different feeding regimes – an initial self-selection/3 way preference phase, a learning/training phase and a final self selection/3-way preference phase.

Naïve self selection/3-way preference phase (7d)

- Each cat received *ad libitum* access to all 3 experimental diets.
- 150g of each diet was offered at 10:15am and was left in the lodge until 8:15am the following morning giving each cat 22 hours exposure to the diets on each day. This feeding cycle was repeated daily for 7 days.
- The position of the diets available was rotated daily.
- Food intakes and feeding patterns were recorded constantly. In addition, food intakes were recorded manually every time food was replaced.

Learning/training phase (24d)

- During the learning/training phase, each cat received a single test diet each day
- The three diets experienced by the cats during the naïve self-selection were fed in daily rotation for the 24-days. Each cat therefore experienced each experimental diet 8 times.
- 150g of a single diet was offered at 10:15am and left in the lodge until 8:15am the following morning. This feeding cycle was repeated daily for 24 days, giving each cat 22 hours exposure to the diet on each day.
- Cats were randomly assigned to one of 6 groups with each group receiving the diets in a different rotation sequence.
- Food intakes and feeding patterns were recorded constantly. In addition, food intakes were recorded manually every time food was replaced.

Experienced self-selection/3-way preference (7d)

- Each cat received *ad libitum* access to all 3 experimental diets.
- See Phase 1
- The position of the diets available was rotated daily.
- Food intakes and feeding patterns were recorded constantly. In addition, food intakes were recorded manually every time food was replaced.

Bodyweights

Bodyweights were recorded twice weekly and closely monitored to ensure adequate food intakes.

Data Analysis

Feeding pattern data were analysed by computer software that splits the data into individual meals, giving time, duration, rate and latency of each parameter. These could then be analysed for each cat and each diet. Overall, 5% of the data was lost for this trial.

Note: During the learning phase, graphs are expressed with 'cycle' on the x-axis. Each 'cycle' is a pseudo-randomised 3-day rotation of products, and therefore includes an exposure to each of the 3 diets.

Results and Data Analysis

Animals

All cats completed the study. The mean percentage change in bodyweight from the start of the trial to the end of the trial was +2.2% averaged over all cats.

Naïve self-selection phase

During the naïve self-selection phase, all 3 test diets were fed every day. All cats received *ad libitum* access to the diets for 22 hours each day. The diets were removed from each lodge at 8:15am every morning and replaced with fresh diet at 10:15am.

The cats had no prior experience of the experimental diets prior to the start of this initial phase.

Figure 13 shows the daily mean food intake, averaged over all cats, for each diet during this 7-day phase. Daily mean intakes of diet B (high protein) and diet F (intermediate) fluctuated throughout the phase but were consistently higher than diet C (high fat).

Figure 14 shows the mean food intake, averaged over all cats, and all days for each diet during the 7-day naïve self-selection phase. On average, the intakes of diet B (high protein) and diet F (intermediate) were significantly higher than diet C (high fat), $p<0.001$

Figure 15 shows the proportion of the total intake of each of the diets for individual cats averaged over all 7 days of the naïve self-selection phase. This shows that the majority of cats follow the pattern shown in Figure 14.

Figure 16 shows the results of using the feeding data to look at the intake pattern of the 3 diets throughout the course of the day. For analysis the day was arbitrarily split into in six 4-hour time blocks. The highest intakes of each diet were during the first 4-hour time block i.e. after food was offered (9am-1pm). The intake for diet C (high fat) remained fairly constant throughout the rest of the day whilst consumption of diet B (high protein) and diet F (intermediate) were more variable . The highest mean intakes were of diet B (high protein) between 5am and 5pm then diet F (intermediate) from 5pm to 5am, however, individual cat intake patterns and daily mean intake patterns show considerable variability in feeding patterns.

Learning Phase

During the monadic learning phase each cat received *ad libitum* access to a single test diet for 22 hours each day, with each group of cats receiving different diets according to cyclic rotation. The diets were removed from each lodge at 8:15am every morning and replaced with fresh diet at 10:15am to allow for cleaning. Food intake data was analysed by 3-day cycle, during which each cat experienced all 3 diets.

Figure 17 shows the daily mean food intake, averaged over all cats, for each diet during each of the 3-day cycles. Intakes for all diets remained fairly constant during the learning phase. Intakes of diet F (intermediate) were marginally higher than the other test diets during cycles 11, 12 and 14 but overall there were no differences in the intake of the three test diets

Figure 18 shows the daily mean food intake, averaged over all cats and all cycles, for each diet during the learning phase. There were no significant differences in the intakes of each diet during this phase.

Figure 19 shows the proportion of total intake of each of the diets for each cat averaged over all cycles of the learning phase. This shows that, during the learning

phase, individual cats followed a similar intake pattern to the overall group mean for each diet.

Figure 20 shows the results of using the data to look at the intake pattern of the 3 diets throughout the course of the day, in 4-hour time blocks. Note: No food was available to the cats between 8.15am and 10.15am each day. Cats were offered one diet per day, unlike the other two phases, for which all three diets were offered simultaneously. A similar pattern of food intake to that seen during the naïve self selection phase was seen during the learning phase such that majority of the high protein and intermediate diets was consumed between 9am and 5pm. The amount of high fat diet consumed between 9am and 1pm was relatively low compared to the other test diets, was similar between 1pm and 5pm and then highest between 5pm and 1am. Individual cat intake patterns and daily mean intake patterns show variability.

Experienced self-selection

During the experienced self-selection phase, all 3 test diets were fed every day. All cats received *ad libitum* access to the diets for 22 hours each day. The diets were removed at 8:15am every morning and replaced with fresh diet at 10:15am to allow for cleaning.

Figure 21 shows the daily mean food intake, averaged over all cats, for each diet during each day. Intakes for diet C (high fat) were consistently low throughout this phase whilst diet B (high protein) remained fairly constant. Intakes of diet F (intermediate) fluctuated daily above and below that of diet B (high protein).

Figure 22 shows the mean intake, averaged over all cats, for each diet during the 7-day experienced self-selection phase. On average, the intakes of diet B (high protein) and diet F (intermediate) were significantly higher than of diet C (high fat), $p<0.001$

Figure 23 shows the proportion of total food intake of each of the diets for individual cats averaged over all days of the experienced self-selection. Proportions vary considerably amongst individuals. Two out of the 12 cats in the study had a markedly higher than average proportional intake of diet C (high fat). The remaining 10 cats almost completely rejected diet C (high fat). Two cats followed the mean intake pattern seen in Figure 22 whilst the rest of the cats had either a high intake of diet B (high protein) or diet F (intermediate).

Figure 24 shows the results of using feeding data to look at the intake pattern of the 3 diets throughout the course of the day, in 4-hour time blocks. Note: No food was available to the cats between 8.15am and 10.15am each day. The highest intakes of each diet were during the first 4-hour block after food is offered. The intake for diet C (high fat) remained fairly constant throughout the rest of the day whilst diets B (high protein) and F (intermediate) fluctuated. The highest mean intakes were of diet B (high protein) between 5am until 5pm then diet F (intermediate) from 5pm to 5am, however, individual cat intake patterns and daily mean intake patterns show considerable variability in feeding patterns.

<u>P/F/CER selection as a potential driver of macronutrient selection</u>

The mean PER intake was calculated for each cat for each phase of the trial from the food intake data.
The calculation used was:

$$\text{Mean PER eaten per day} = \frac{\sum_{\text{Over all 3 test diets}} (\text{Amount of test diet eaten (g)} \times \text{PER of test diet})}{\text{Total amount eaten (g)}\,*}$$

* For self-selection phase (naïve/experienced) = sum of 3 test diets eaten per day (g)
For learning phase = sum of 3 test diets eaten over 3-day cycle (g)

The daily/per cycle mean PER intake was calculated for each cat. This was repeated for FER.

Note: CER values were 25% for all calculations performed and are excluded from the discussions below.

Table 3 shows the mean daily/cycle PER and FER for each phase of the trial, averaged over all cats. If random sampling had taken place and thus equal amounts of each diet were eaten, the expected PER/FER would be 35%/40%. Energy ratios during the learning phase were very close to random sampling values as the g intake for each diet were very similar (See Table 4 for g intake). Energy ratios during the naïve self-selection were closer to random sampling values than during the experienced self-selection. PER was higher and FER was lower than random sampling in the naïve and experienced phases.

Table 3: Mean cycle PER, FER and CER intake for each phase

	Naïve self-selection	Learning	Experienced self-selection
PER	36.7%	34.7%	39%
FER	38.4%	40.4%	36%

Table 4: Mean daily intake (g) of each diet during each phase

	Diet B (**High Protein**)	**Diet C** (**High Fat**)	**Diet F** (**Intermediate**)
Naïve self-selection (3 diets offered)	19.2	10.9	21.5
Learning (one diet offered)	51.9	51.6	55.8
Experienced self-selection (3 diets offered)	26.0	6.4	25.0

Figure 25 shows the mean P/FER for each cycle during each phase of the trial. PER was lower than FER during the naïve self-selection for each cycle (day), except the 3rd when both were about equal. PER intake was lower and FER was higher during the monadic learning phase than during the naïve self selection phase and remained relatively constant during each 3-day cycle of the monadic learning phase. There was a switch during the experienced self-selection such that PER was higher than FER for all cycles (days) except 19 and 20 when they were comparable.

Conclusions

Group analysis of cycle mean intake showed that the high protein (48%PER/26%FER) and intermediate diets (34%PER/42%FER) were equal before, during and after a period of monadic learning. This suggests that, on average, these diets are equal in preference in terms of hedonics and macronutrient profile. Analysis of individual cat response showed a distinctive shift in preference in the majority of experienced cats towards one or the other of these diet.

Mean cycle intakes of the high fat diet (22%PER/53%FER) were considerably lower than the other diets during the self-selection phases when all 3 diets were offered. There was a decrease in intake of the diet after a period of monadic learning suggesting that the macronutrient profile was less desirable than the other diets.

Food intakes of all 3 diets were equal during the monadic learning phase when each diet was offered individually each day. Cats were therefore willing to eat the high fat diet if no other option was available. Investigation of feeding pattern during the monadic learning phase showed that the cats didn't eat the high fat diet as rapidly as the other test diets after they were offered in the morning. This could be that the cats were holding out for another option on the days that they were offered the high fat diet and then eating it when no other diets were offered. Alternatively they were regulating the rate at which fat was consumed.

Example 4

Effect of macronutrient profile on the long-term acceptance of food:
Effect of varying Carbohydrate and Fat Energy Ratios

Summary

12 adult cats were fed 3 diets (one high in carbohydrate, one high in fat and one intermediate). This study the followed the following feeding regime: 7-days of self-selection/ 3-way preference followed by 24-days of monadic learning (one diet per day) and finally 7 days of self-selection/3-way preference. Cats had approximately 22 hours access to food every day during the trial.

Of the 12 cats allocated, 11 completed the study.
One cat was withdrawn from trial in the 3rd week due to low intakes in the self-selection phase and frequent refusals in the monadic phase. Data from this cat has not been calculated in the means.

The mean percentage change in bodyweight during the trial, for the cats that completed the study was –1.6%.

The 1st phase response of the cats to the 3 diets was that the high carbohydrate and intermediate diets were preferred (mean intakes 18.4g and 14.3g respectively) over the high fat diet (mean intake 9.3g). It should be noted however, that the intake of intermediate fluctuated and the intake of the high fat diet showed a marked upturn at the 7th cycle, which may indicate that a change of preference was taking place.

The experienced response of the cats to the 3 diets was very different to that seen during the naïve self-selection. High fat diet was preferred (mean intake 57.3g) to the high carbohydrate and intermediate (mean intakes 3.1g and 7.5g respectively.

Investigation of the mean proportion energy intakes of protein and fat (PER/FER) showed cats consumed 24.4% PER, 34.1% FER in the naïve self-selection phase. The same analysis of PER/FER during the experienced self-selection showed the mean proportion of macronutrients consumed to be 26.9% PER, 50.7% FER in the experienced self-selection phase.

In summary, the high fat diet appeared to be hedonically more palatable than the high carbohydrate and intermediate diets. The increased preference for the high fat diet against the decrease in CER and PER during the experienced self-selection (+14.6% and –2% respectively), provides evidence that the macronutrient profile of the high fat (22%PER, 54%FER, 24%CER) diet is preferred to the intermediate (24%PER, 38%FER, 38 %CER) and high carbohydrate (26%PER, 21%FER, 53%CER) diets.

Introduction

The aim of this study was to assess diets with similar protein energy ratios (PER) but variable carbohydrate and fat energy ratios. One diet had a high fat energy ratio (FER), another had a high carbohydrate energy ratio (CER) and a third diet had an intermediate energy ratio of carbohydrate and fat.

Methodology

Animals

Cats (n = 12) were selected that had been fed dry kibble diets throughout life, from the weaning.

The cats were housed individually and were socialised as a group every day.

Diets

Three dry kibble diets were fed during the study. The diets were designed to all contain the same level of protein whilst one diet was enriched with carbohydrate, one enriched with fat and a third was the intermediate of the other two diets. Analysis of the diets provided the predicted metabolisable energy (PME) content of each diet, the values of which are shown in Table 1. The protein, carbohydrate and fat content of each diet was analysed and calculated to provide the ratio of each macronutrient relative to the total energy (PME) of each diet i.e. Protein/Fat/Carbohydrate Energy Ratio (P/F/CER in Table 1).

Table 1: PME and macronutrient energy ratios for BS0114 diets

Diet Code	% expected PER:FER:CER	PME (Kcal/100g)
A (High Carbohydrate)	26 / 21 / 53	346
D (Intermediate)	24 / 38 / 38	390
C (High Fat)	22 / 54 / 24	436

Feeding Protocols

The feeding protocol used is set out in Example 2.

Bodyweights

Bodyweights were recorded twice weekly and closely monitored to ensure adequate food intakes.

Data Analysis

Is as set out in Example 2.

Results and Data Analysis

Animals

Of the 12 cats that started, 11 cats completed the study.
The mean percentage change in bodyweight from the start of the trial to the end of the trial was –1.6% averaged over all cats.

Figure 26 shows the mean intakes throughout the trial.

This chart shows the mean intakes throughout the trial. The 3 phases are identified as:

Naïve = cycles 1 to 7

Learning = cycles 8 to 15

Experienced = cycles 16 to 22.

It can seen quite clearly that the cats having sampled all the diets during the naïve phase of self-selection, then proceeded to make a clear choice of the high fat diet over the intermediate and high carbohydrate diets in the remaining 2 phases.

P/F/CER selection as a potential driver of macronutrient selection

The mean FER intake was calculated as set out in Example 2.

If random sampling had taken place and thus equal amounts of each diet were eaten, the expected PER/FER/CER would be 24% /38% / 38%.

Table 3 shows the mean cycle PER, FER and CER intake.

Table 3: Mean cycle PER, FER and CER intake for each phase.

	Phase 1 Naive self-selection	Phase 2 Learning/Monadic	Phase 3 Experienced self-selection
PER (%)	19.5	18.5	17.7
FER (%)	34.7	42.3	49.4
CER (%)	19.5	39.3	32.9

During the naïve phase all 3 energy ratio intakes were less than would be expected from random sampling.

FER and CER ratios during the learning phase were greater and PER was less than expected if random sampling was taking place. .

During experienced self-selection FER was greater and both PER and CER were less than expected if random sampling was taking place.

Table 4: Mean daily intake (g) of each diet for each phase

	Phase 1 Naive self-selection (3 diets offered)	Phase 2 Learning/Monadic (one diet offered)	Phase 3 Experienced self-selection (3 diets offered)
Diet A (High CHO)	18.4	32.9	3.1
Diet C (High Fat)	9.3	68.7	57.3
Diet D (Intermediate)	14.3	51.6	7.5

Table 5: Mean daily intake (g) of macro-nutrient for each phase

	Phase 1 Naïve self-selection	Phase 2 Learning/Monadic			Phase 3 Experienced self-selection
		Diet A	Diet C	Diet D	
Protein (g/day)	8.9	6.9	10.6	14.9	14.6
Fat (g/day)	6.7	3.1	9.1	18.2	16.8
CHO (g/day)	20.6	18.1	24.8	26.3	27.3

Tables 4 & 5 show the mean intakes in grams of the diets and the macronutrients, through the three phases of the trial. These also show that the high carbohydrate diet was rejected after the initial experiences of the naïve phase, in favour of the high fat diet.

Figure 27 shows the mean C/FER for each cycle during each phase of the trial. FER was lower than CER during the naïve self-selection for each cycle (day).
CER intake was lower and FER was higher during the monadic learning phase than during the naïve self-selection phase and remained relatively constant during each 3-day cycle of the monadic learning phase.

During the experienced self-selection FER was higher than CER for all cycles (days).

This suggests that during the naïve phase the cats were learning that there was a difference between the 3 diets, and that in the following two phases they were actively seeking out the fat enriched diet in preference to the other two diets.

Conclusions

Analysis of cycle mean intakes showed that the high fat diet was consistently preferr to the other two diets, after the initial sampling in the naïve phase. This suggests that selection is taking place and not merely a random sampling of diets.

Mean cycle intakes of the high fat diet were considerably higher than the other diets during both the learning monadic and the experienced self-selection phases. There wa a decreased intake of the high carbohydrate and intermediate diets indicating they were less preferred to the high fat diet.

Previous trials show that cats will reject diets with a PER of 20% or less after a perio of monadic learning. The PER was a constant in this trial at 24% and as such has no impact on the macronutrient selection.

Analysis of feeding patterns showed that intake of the high carbohydrate and intermediate diets, in the naïve phase, was greater in the time slots between 9:00am and 5:00pm. After this time all intakes were similar.

During the monadic learning phase the intake of the high fat diet was higher and the pattern also showed an upturn of intake in the 1:00am – 5:00am time-slot. This suggests that the cats had learnt that the next meal would be a less preferred diet and were compensating for that.

Investigation of feeding patterns during the monadic learning phase showed that the cats didn't eat the high carbohydrate diet as readily as the other test diets. This suggests that the cats were holding out for another option.

Example 5

Effect of macronutrient profile on the long-term acceptance of food:
Effect of varying Carbohydrate and Protein Energy Ratios

Summary

12 adult cats were fed 3 diets (one high in carbohydrate, one high in protein and one intermediate). This study the followed the following feeding regime: 7-days of self-selection/ 3-way preference followed by 24-days of monadic learning (one diet per day) and finally 7 days of self-selection/3 way preference. Cats had approximately 22 hours access to food every day during the trial. All 12 cats completed the trial.

The mean percentage change in bodyweight during the trial, for the cats that completed the study was +2.3%.

The 1st phase response of the cats to the 3 diets was that the high protein was preferred (mean intake 41g) over the intermediate and high carbohydrate diets (mean intake 22.7g & 5.1g respectively). It should be noted however, that the intake of intermediate was higher than high protein on day 1 and they were the same on day 4.

The experienced response of the cats to the 3 diets was very similar to that seen during the naïve self-selection. High protein diet was preferred (mean intake 49.7g) to the high carbohydrate and intermediate (mean intakes 2.9g and 21.6g respectively). As in the naïve phase it should be noted however, that the intake of intermediate and high protein on day 1 were similar and again on day 4.

Investigation of the mean proportion energy intakes of protein and carbohydrate (PER/CER) showed cats consumed 28.8% PER, 21.6% CER in the naïve self-selection phase. The same analysis of PER/CER during the experienced self-selection showed

the mean proportion of macro-nutrients consumed to be 32.1% PER, 22.8% CER in the experienced self-selection phase.

In summary, the high protein diet appeared to be hedonically more palatable than the high carbohydrate and intermediate diets during both the naïve and the experienced self-selection .

This indicates that the macronutrient profile of the high protein diet (51%PER,24%FER,25%CER) is preferred to both the intermediate (37%PER, 23%FER,40 %CER) and high carbohydrate (26%PER, 21%FER, 53%CER) diets. However, during the monadic learning phase there was little distinction between high protein and intermediate diets. The high carbohydrate was still rejected.

<u>Introduction</u>

The aim of this study was to assess diets with similar fat energy ratios (FER) but variable carbohydrate and protein energy ratios. One diet had a high protein energy ratio (PER), another had a high carbohydrate energy ratio (CER) and a third diet had an intermediate energy ratio of carbohydrate and protein.

<u>Methodology</u>

Animals

Cats (n = 12) were selected that had been fed dry kibble diets throughout life, from the weaning.

The cats were housed individually and were socialised as a group every day.

Diets

Three dry kibble diets were fed during the study. The diets were designed to all contain the same level of fat whilst one diet was enriched with carbohydrate, one enriched with protein and a third was the intermediate of the other two diets. Analysis of the diets provided the predicted metabolisable energy (PME) content of each diet, the values of which are shown in Table 1. The protein, carbohydrate and fat content of each diet was analysed and calculated to provide the ratio of each macronutrient relative to the total energy (PME) of each diet i.e. Protein/Fat/Carbohydrate Energy Ratio (P/F/CER in Table 1).

Table 1: PME and macronutrient energy ratios for diets

Diet Code	% expected PER:FER:CER	PME (Kcal/100g)
A (High Carbohydrate)	26 / 21 / 53	348
B (High Protein)	51 / 24 / 25	333
E (Intermediate)	37 / 23 / 24	336

Feeding Protocols

- The feeding protocol used is set out in Example 2.

Bodyweights

Bodyweights were recorded twice weekly and closely monitored to ensure adequate food intakes.

Data Analysis

Is as set out in Example 2.

Results and Data Analysis

Animals

All of the 12 cats completed the study.
The mean percentage change in bodyweight from the start of the trial to the end of the trial was +2.3% averaged over all cats.

Figure 28 shows the mean intakes throughout the trial.

This chart shows the mean intakes throughout the trial. The 3 phases are identified as:

Naïve = cycles 1 to 7
Learning = cycles 8 to 15
Experienced = cycles 16 to 22.

It is quite clear that the cats having sampled all the diets during the naïve phase of self-selection, rejected the carbohydrate diet. The preferred diet was the high protein in the remaining 2 phases. However in the monadic learning phase the intermediate diet was equally accepted as the high protein.

P/F/CER selection as a potential driver of macronutrient selection

The mean PER intake was calculated as set out in Example 2.

Thus the daily/per cycle mean PER / FER / CER intake was calculated. (See table 3)

If random sampling had taken place and thus equal amounts of each diet were eaten, the expected PER/FER/CER would be 38% / 23% / 39%.

Table 3: Mean cycle PER, FER and CER intake for each phase

	Phase 1 Naive self-selection	Phase 2 Learning/Monadic	Phase 3 Experienced self-selection
PER (%)	43.5	39.9	44.7
FER (%)	23.6	23.9	23.5
CER (%)	32.9	36.2	31.8

Throughout all three phases FER intakes were as would be expected from random sampling.

PER were greater and CER were less than expected if random sampling was taking place.

This indicates that selection was being made rather than random sampling of the diets.

Table 4: Mean daily intake (g) of each diet for each phase

	Phase 1 Naive self-selection (3 diets offered)	Phase 2 Learning/Monadic (one diet offered)	Phase 3 Experienced self-selection (3 diets offered)
Diet A (High CHO)	5.1	37.9	3.9
Diet B (High Protein)	41.0	73.9	49.8
Diet E (Intermediate)	22.7	72.1	21.9

Table 5: Mean daily intake (g) of macro-nutrient for each phase

	Phase 1 Naive self-selection	Phase 2 Learning/Monadic			Phase 3 Experienced self-selection
		Diet A	Diet B	Diet E	
Protein (g/day)	28.8	9.0	27.2	34.2	32.2
Fat (g/day)	6.4	3.9	6.9	6.7	7.0
CHO (g/day)	21.6	19.2	25.3	19.9	23.0

Tables 4 & 5 show the mean intakes in grams of the diets and the macro-nutrients, throughout the three phases of the trial. These also show that the high carbohydrate diet was rejected throughout the trial.

Table 6: Proximate dietary analysis and energy ratios

	Proximate analysis (g or kcal/100g)				Energy Ratio (%)		
	Protein (g)	Fat (g)	CHO (g)	PME (kcal)	PER	FER	CER
Diet A (High CHO)	23.8	10.3	50.6	348.0	26	21	53
Diet B (High Protein)	46.3	9.0	26.9	332.7	51	24	25
Diet E (Intermediate)	37.7	9.6	35	336.1	37	23	40

Figure 29 shows the mean C/PER for each cycle during each phase of the trial. CER was lower than PER through each cycle, in all three phases.
This suggests that the cats identified and rejected the high carbohydrate from the beginning.

Conclusions

Analysis of cycle mean intakes showed that the high protein and intermediate diets were consistently preferred to the high carbohydrate diet. This suggests that selection is taking place and not a random sampling.

The high protein diet appeared to be hedonically more palatable than the high carbohydrate and intermediate diets during both the naïve and the experienced self-selection.

Mean cycle food intakes indicate that the macronutrient profile of the high protein diet (51%PER,24%FER,25%CER) is preferred to both the intermediate (37%PER, 23%FER,40 %CER) and high carbohydrate (26%PER, 21%FER, 53%CER) diets during naïve and experienced self selection phases. However, during the monadic learning phase there was little distinction between high protein and intermediate diets. The high carbohydrate was rejected throughout all three phases.

The high carbohydrate diet (CER 53%) had a PER of 26% but was still rejected over the other two diets. As the FER was similar for all three diets, it would indicate that the cats are making selection based on PER.

Analysis of feeding patterns in phase 1 the intake for high protein (diet B) is most preferred, followed by intermediate (diet E). The high carbohydrate diet is consistently rejected throughout the day.

In phase 2 when the cats have no choice, they will eat all the diets but the preference is fluctuating between high protein and intermediate diets, although at the time-slots 5pm-1am there is almost parity across the 3diets.

In phase 3 the high protein diet is clearly preferred to the other two diets.
The highest mean intakes are between 9am and 5pm, presumably stimulated by outside influences of the working day.

Claims

1. A multi-component foodstuff comprising two or more compartmentalised food compositions, of which at least two compositions differ in their content of at least two of fat, protein or carbohydrate.

2. A foodstuff, as claimed in claim 1, wherein the difference in the content of at least two of fat, protein and carbohydrate is of at least 1% on an energy ratio basis.

3. A foodstuff, as claimed in claim 1 or claim 2, wherein the difference in fat content is of from 1 to 40% on a fat:energy ratio.

4. A foodstuff, as claimed in any one of claims 1 to 3, wherein the difference in protein content is of from 1 to 40% on a protein:energy ratio.

5. A foodstuff, as claimed in any one of claims 1 to 4, wherein the difference in carbohydrate content is of from 1 to 40% on a carbohydrate:energy ratio.

6. A foodstuff, as claimed in any one of claims 1 to 5, wherein at least one component is a dried ready-to-eat cereal product.

7. A foodstuff, as claimed in any one of claims 1 to 6, wherein one component comprises at least 40% fat on an energy ratio basis and a different component comprises at least 40% protein on an energy ratio basis.

8. A foodstuff, as claimed in any one of claims 1-7, for use in providing an optimum macronutrient diet to an animal.

9. A foodstuff, as claimed in any one of claims 1-7, for use in animal weight maintenance.

10. A foodstuff, as claimed in any one of claims 1-7, for use in animal health benefit.

11. A foodstuff, as claimed in any one of claims 1-10, wherein the food compositions are separately packaged.

12. Food composition, for use in a foodstuff as claimed in any one of claims 1-11.

13. A method of providing an optimum macronutrient diet to an animal, the method comprising:

feeding said animal a multi-component foodstuff, as set out in any one of claims 1-7.

14. A method of animal weight maintenance, the method comprising:

feeding said animal unlimited quantities of a multi-component foodstuff as set out in any one of claims 1-7.

15. A method of promoting animal health, the method comprising:

feeding said animal a multi-component foodstuff as set out in any one of claims 1-7.

Figure 1

Dry Diets of Varying Macronutrient Profiles

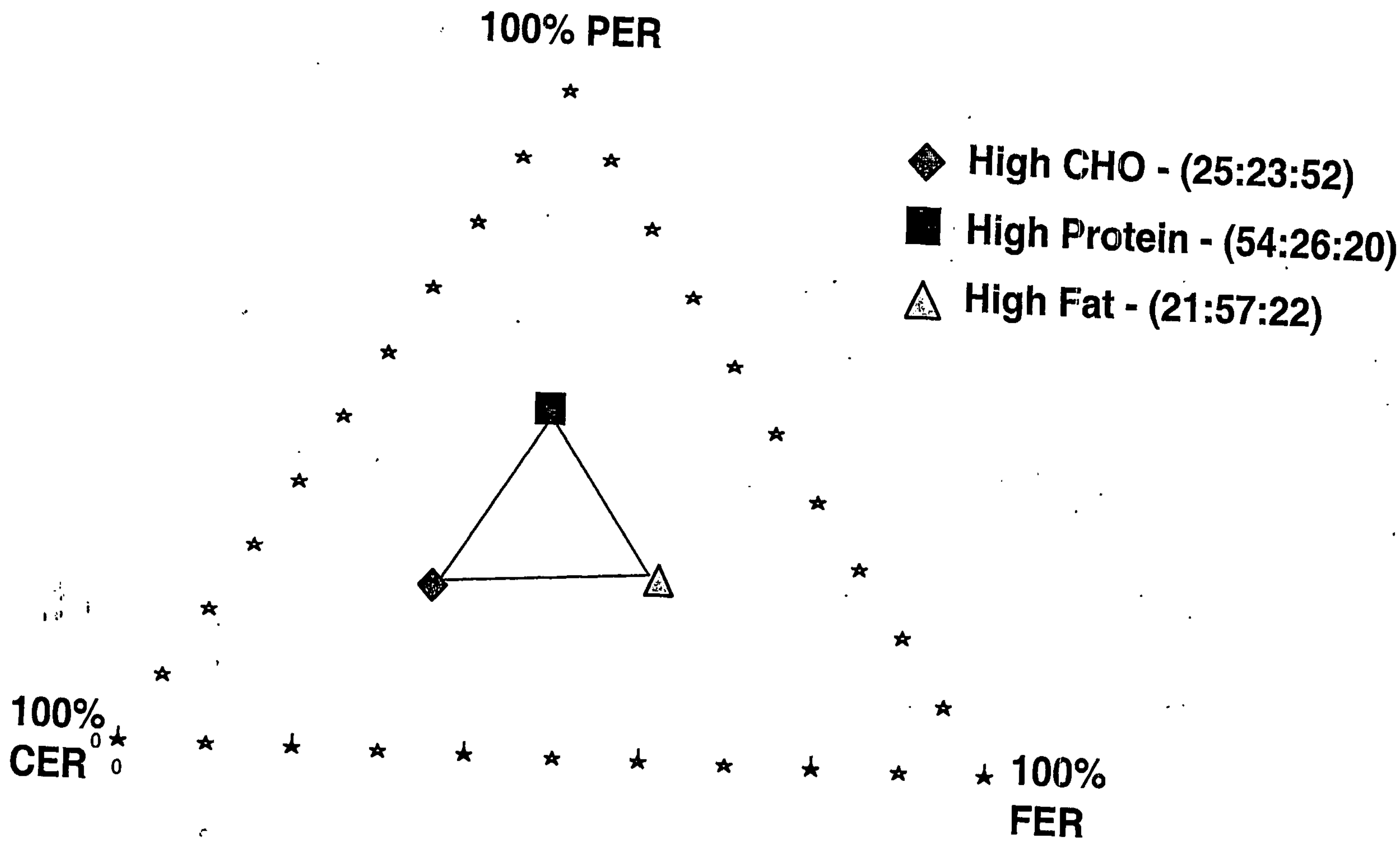


Figure 2: Soy Isolate/lard study

As shown: in days 1 to 7, naïve cats selected diet with preferred flavour regardless of nutrinet profile.

After monadic training period (days 65 to 71), same cats consistently rejected low protein/high fat food regardless of flavour added.

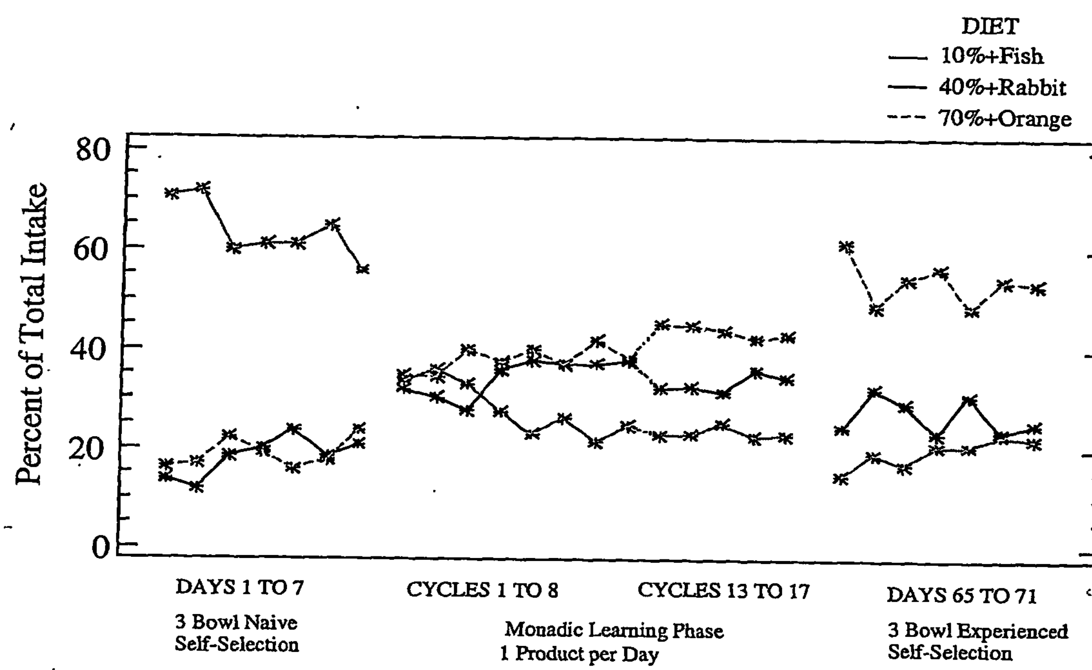


Figure 3: Mean percentage bodyweight change during the trial

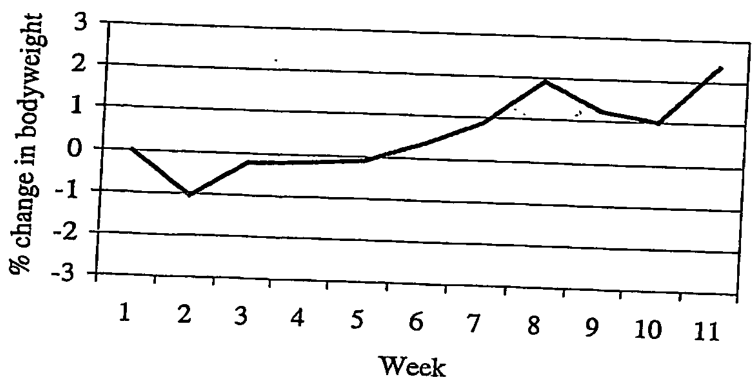


Figure 4: Daily Mean intake, averaged over all cats, for each diet during the naïve self selection

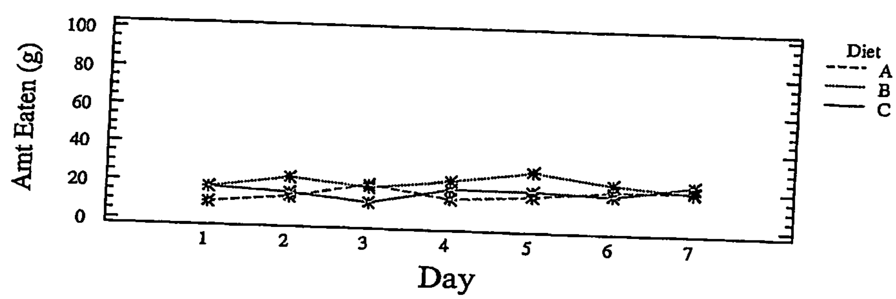


Figure 5: Mean daily intake, averaged over all cats and all days, for each diet during naïve self selection

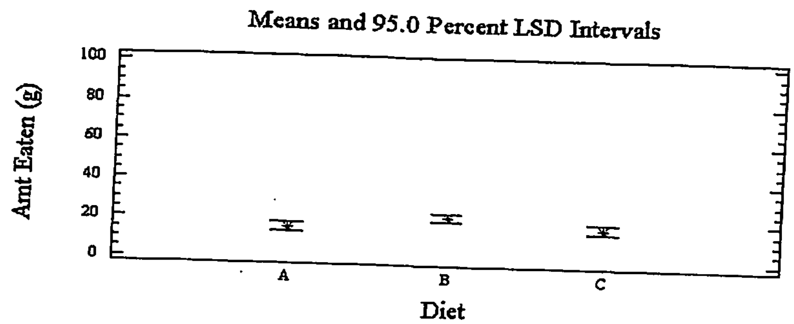


Figure 6: Proportion of total eaten of each diet, averaged over all days, for each cat during naïve self – selection

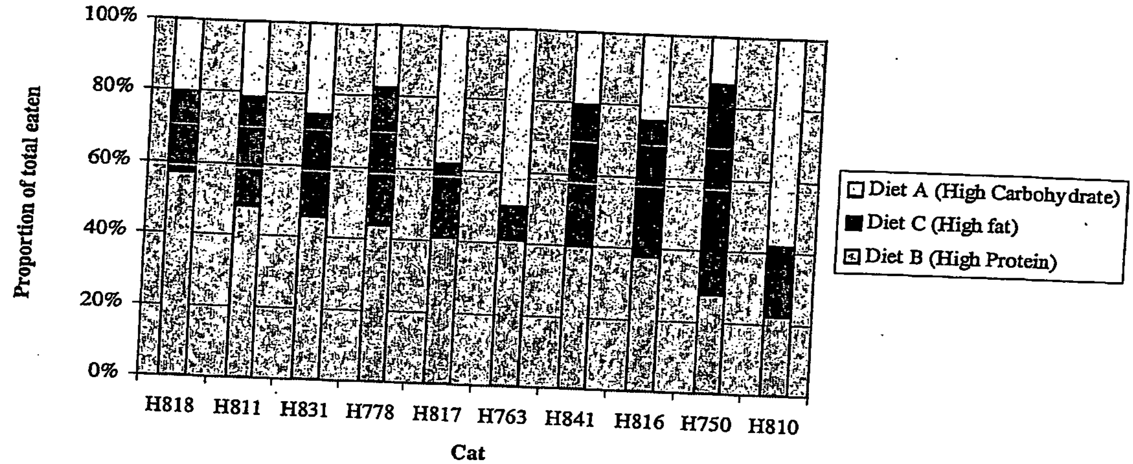


Figure 7: Daily mean intake, averaged over all cats, for each diet during each of the eight 3-day cycles

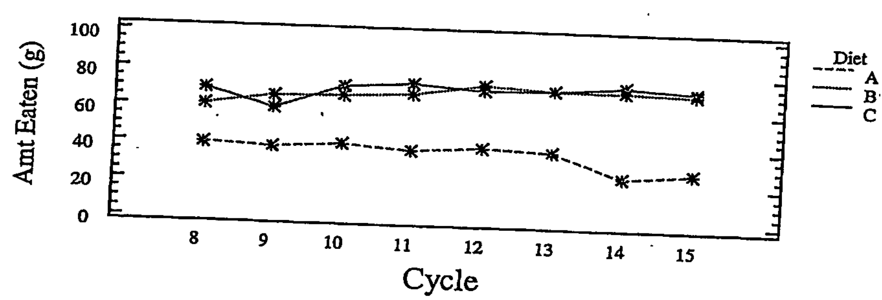


Figure 8: Daily mean intake, averaged over cats and all cycles, for each diet during the learning phase (including results from naïve self-selection)

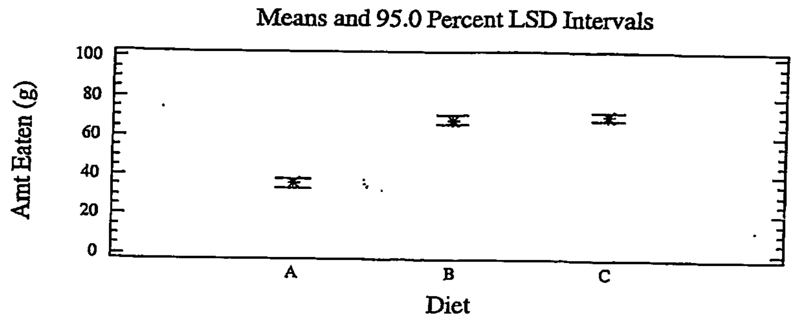


Figure 9: Daily mean intake, averaged over all cats, for each diet during experienced self-selection

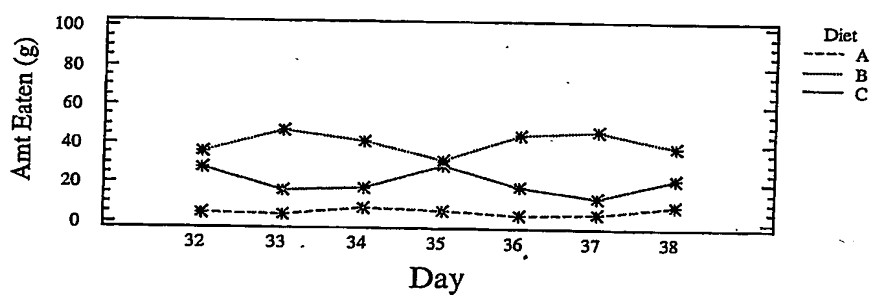


Figure 10:Mean Daily intake, averaged over all cats and all days, for each diet during experienced self-selection

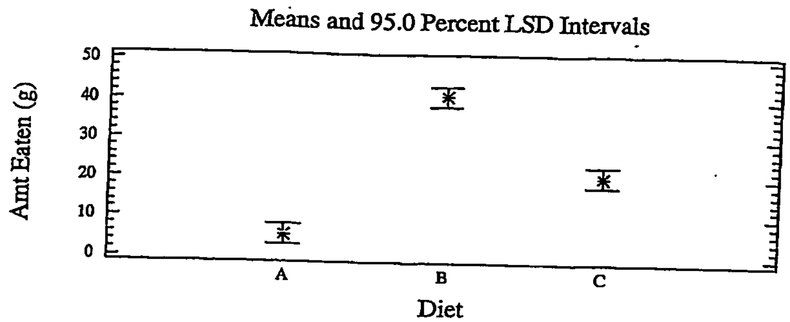


Figure 11: Proportion of total eaten of each diet, averaged over all days, for each cat during experienced self-selection

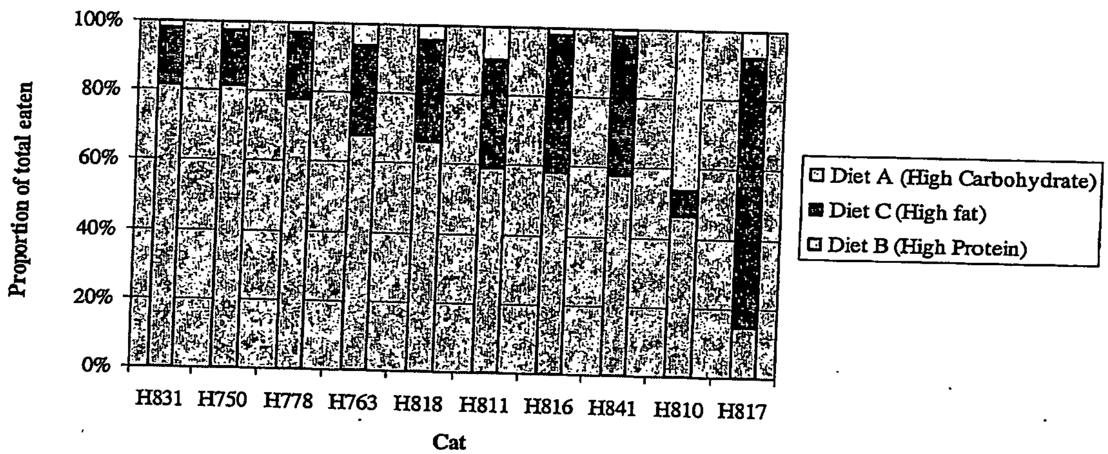


Figure 12: Mean daily P/F/CER intake during the trial

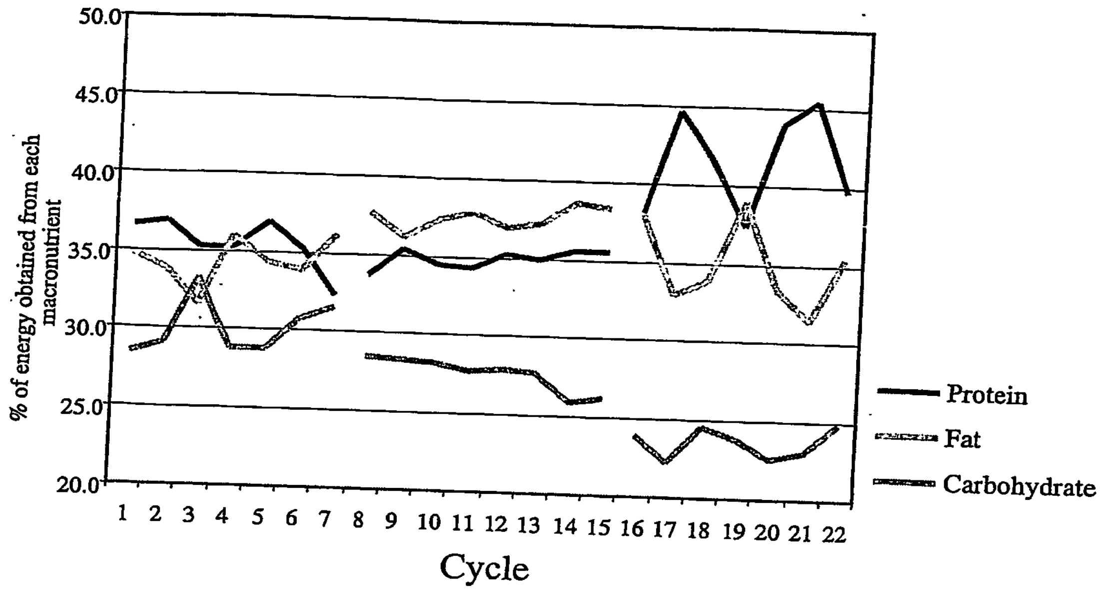


Figure 13: Daily mean food intake, averaged over all cats, for each diet during the naïve self selection phase

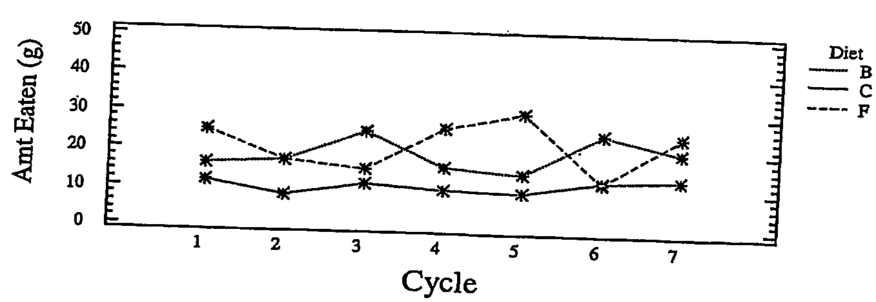


Figure 14: Mean intake, averaged over all cats and all days, for each diet during naïve self – selection

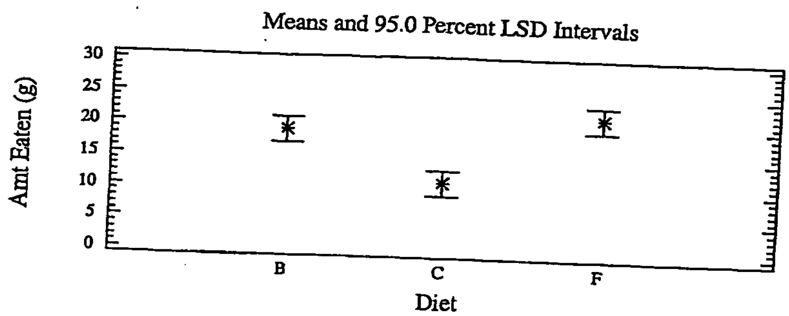


Figure 15: The amount of each diet consumed as a proportion of the total food eaten, averaged over all days, for each cat during the naïve self – selection phase

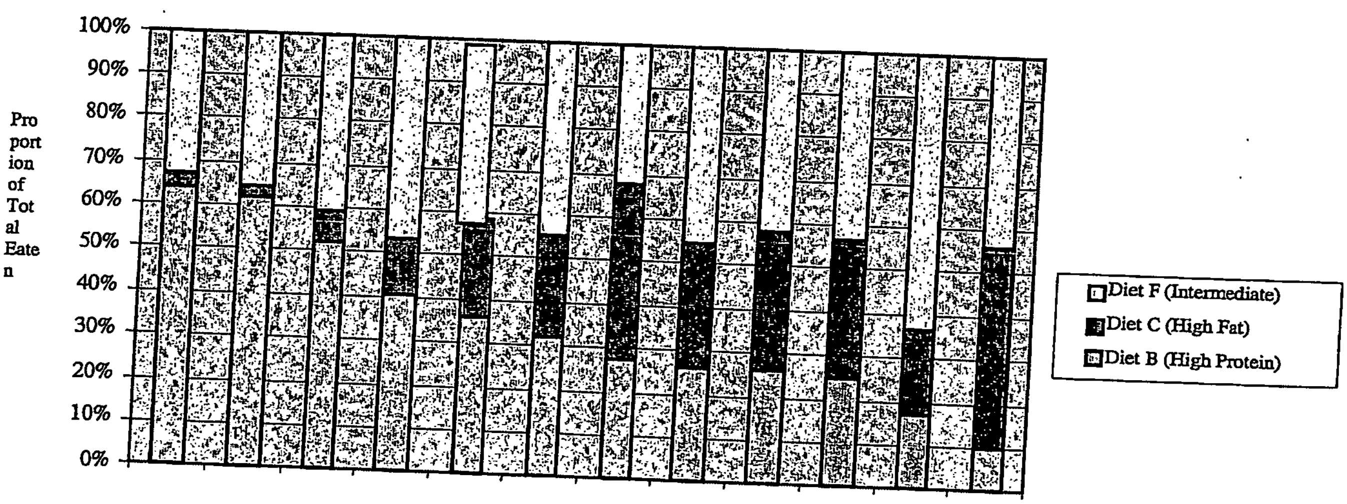


Figure 16: Mean daily food intake pattern for the 3 diets throughout the course of the day (split into 4-hour time blocks) – naïve self selection phase

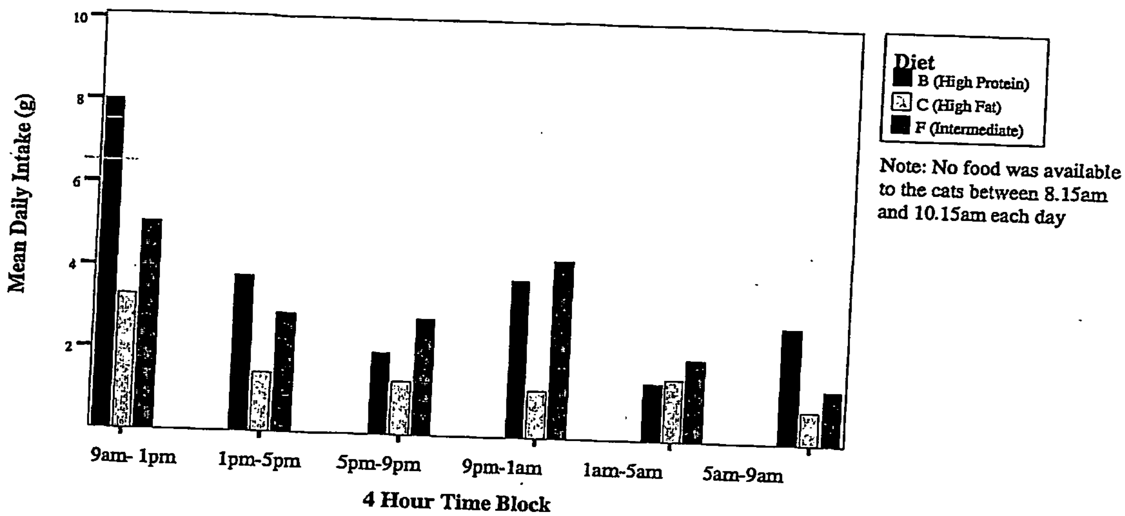


Figure 17: Daily mean food intakes, averaged over all cats, for each diet during each of the eight 3-day cycles

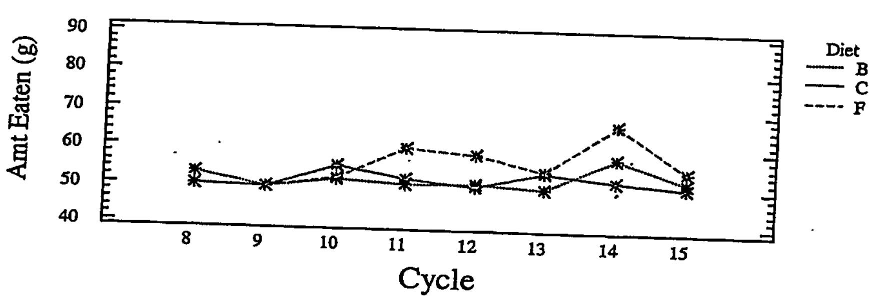


Figure 18: Daily mean food intake, averaged over cats and all cycles, for each diet during the learning phase

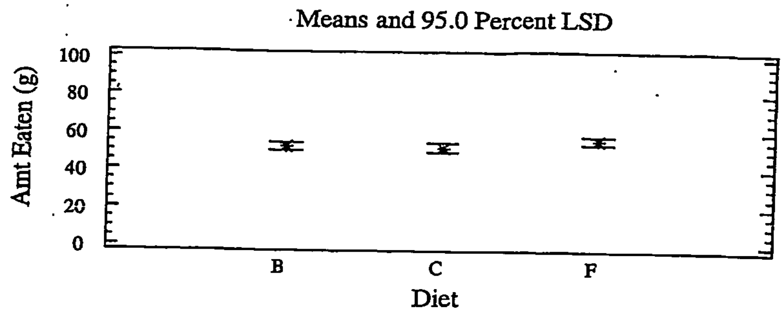


Figure 19: The amount of each diet consumed as a proportion of the total food eaten, averaged over all days, for each cat during the learning phase

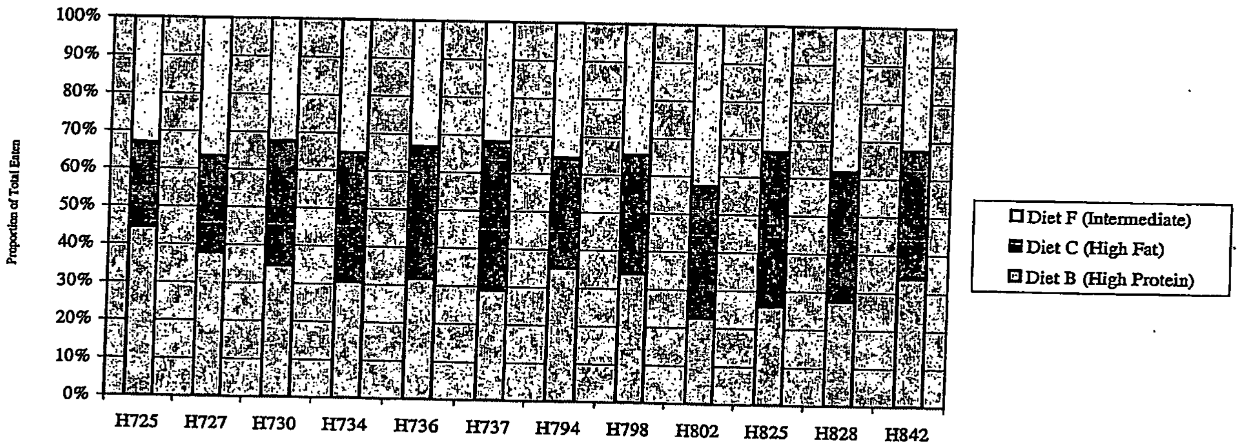


Figure 20: Mean daily food intake pattern for the 3 diets throughout the course of the day (split into 4-hour time blocks) – learning phase

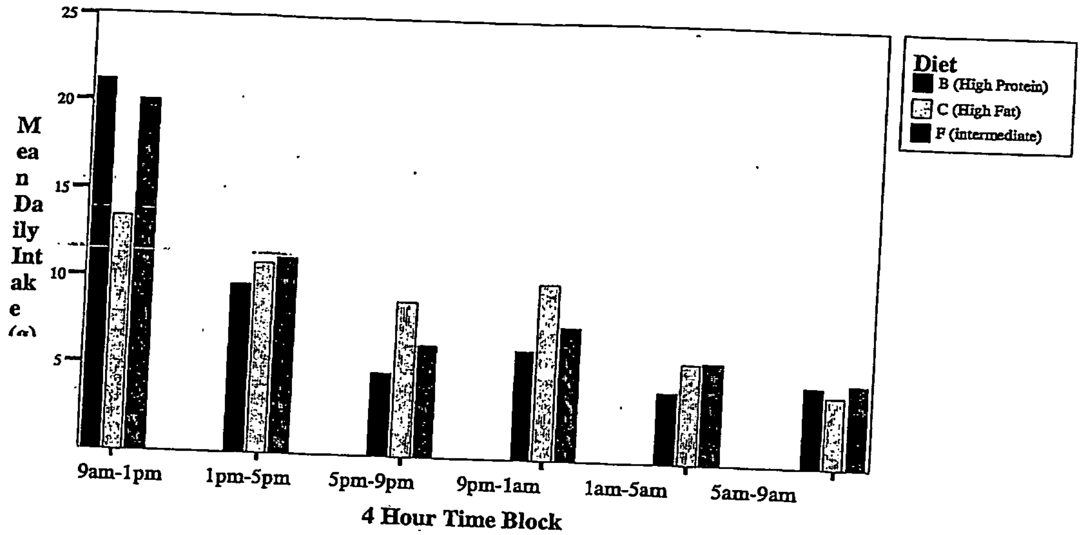


Figure 21: Daily mean food intake, averaged over all cats, for each diet during experienced self-selection phase

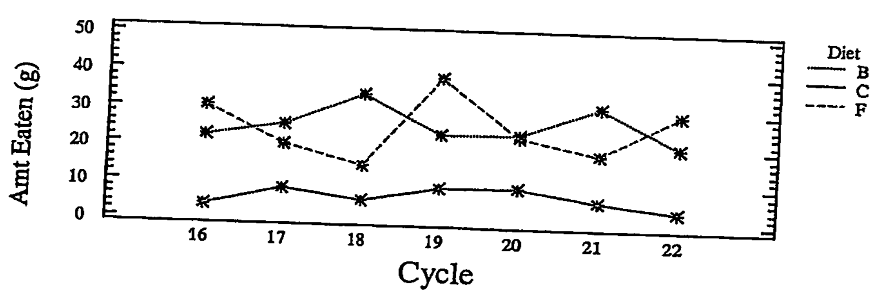


Figure 22: Daily mean food intake, averaged over all cats and all days, for each diet during the experienced self-selection phase

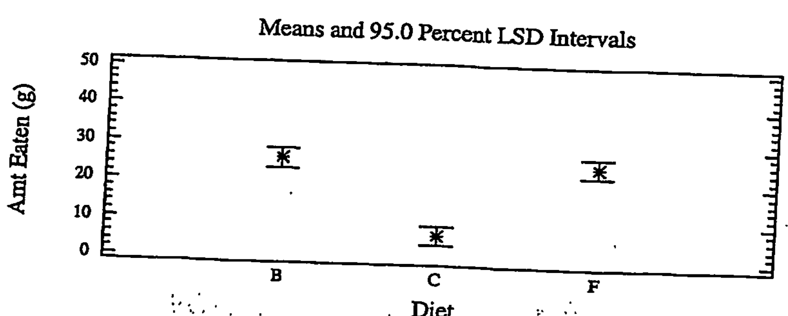


Figure 23: The amount of each diet consumed as a proportion of the total food eaten, averaged over all days, for each cat during the experienced self selection phase

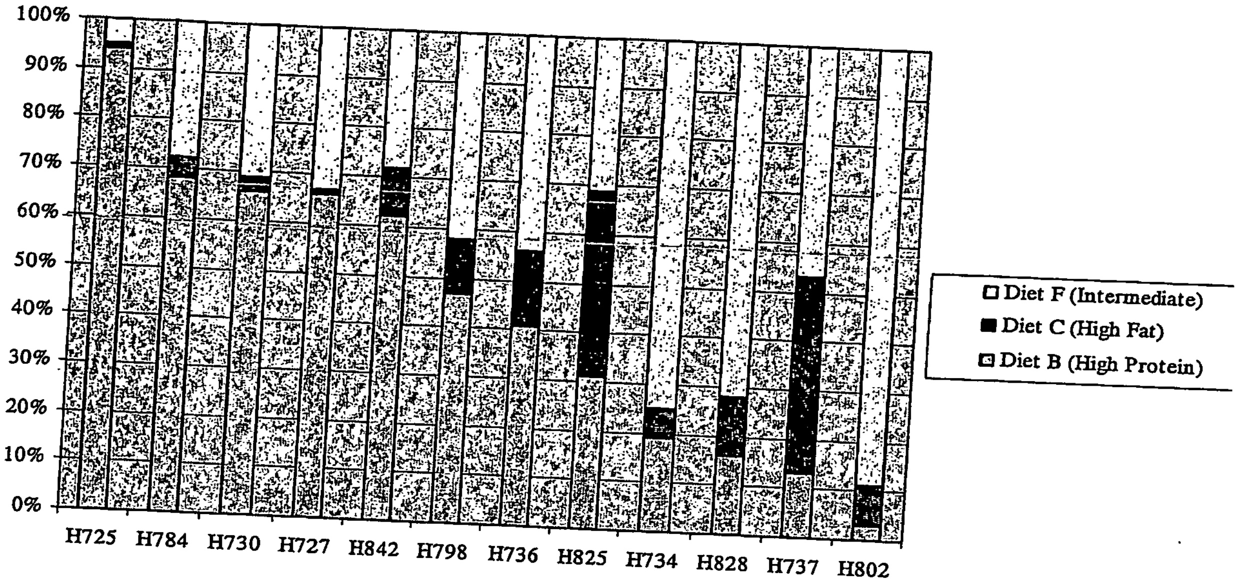


Figure 24: Mean daily food intake pattern of the 3 diets throughout the course of the day (split into 4-hour time blocks) – experienced self selection phase

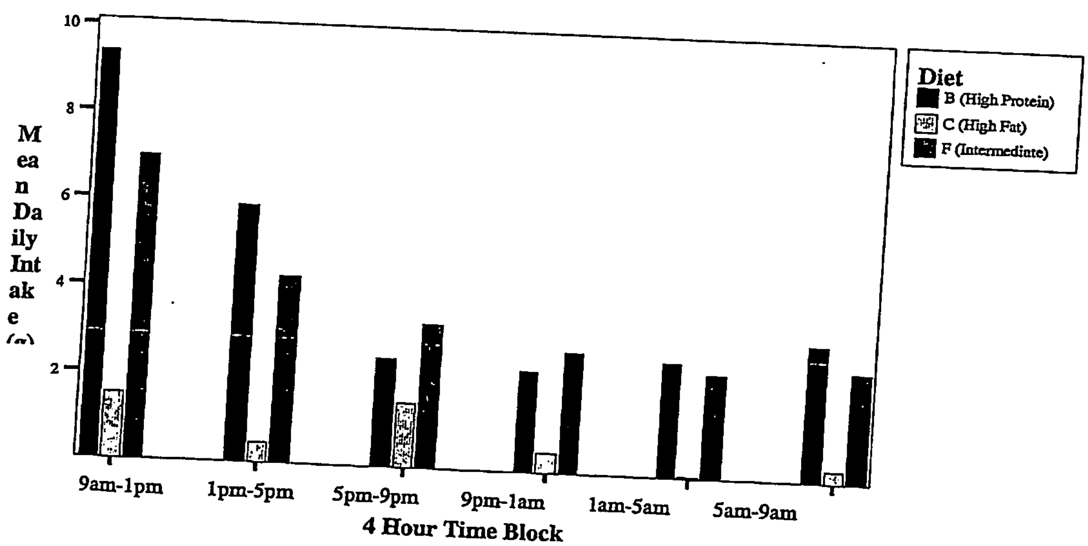


Figure 25: Mean daily P/FER intake during the trial

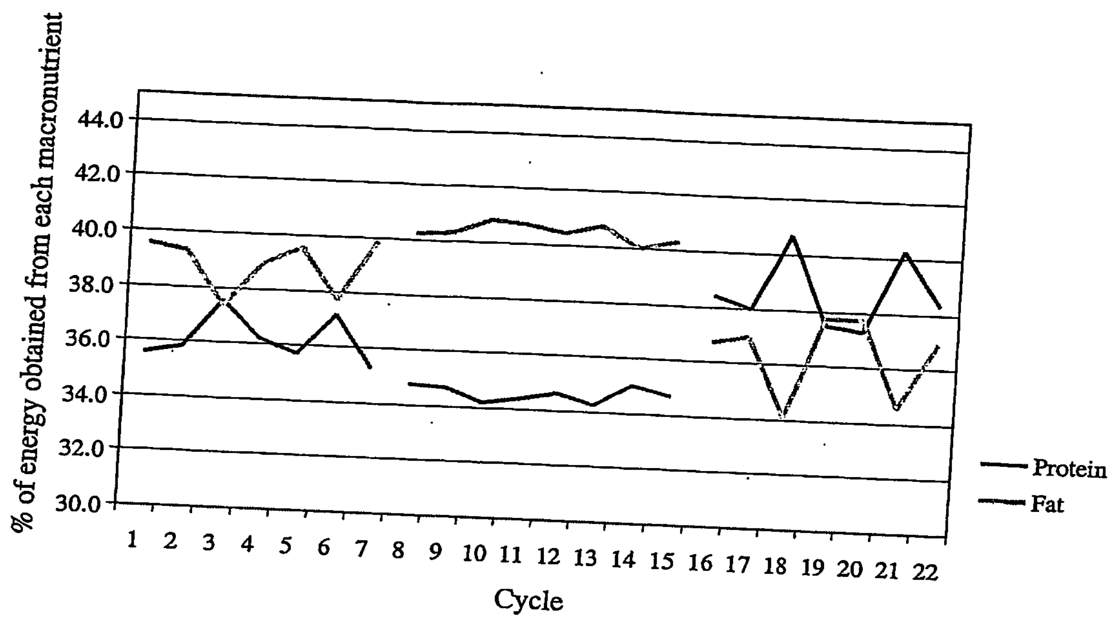


Figure 26: Mean Intakes throughout the trial.

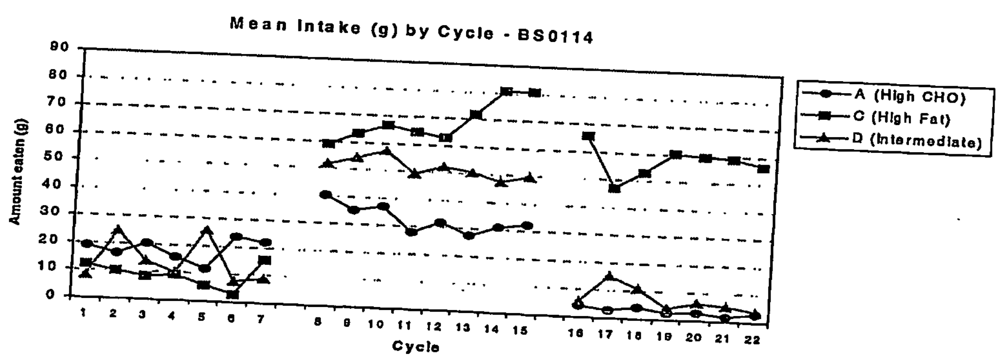


Figure 27:

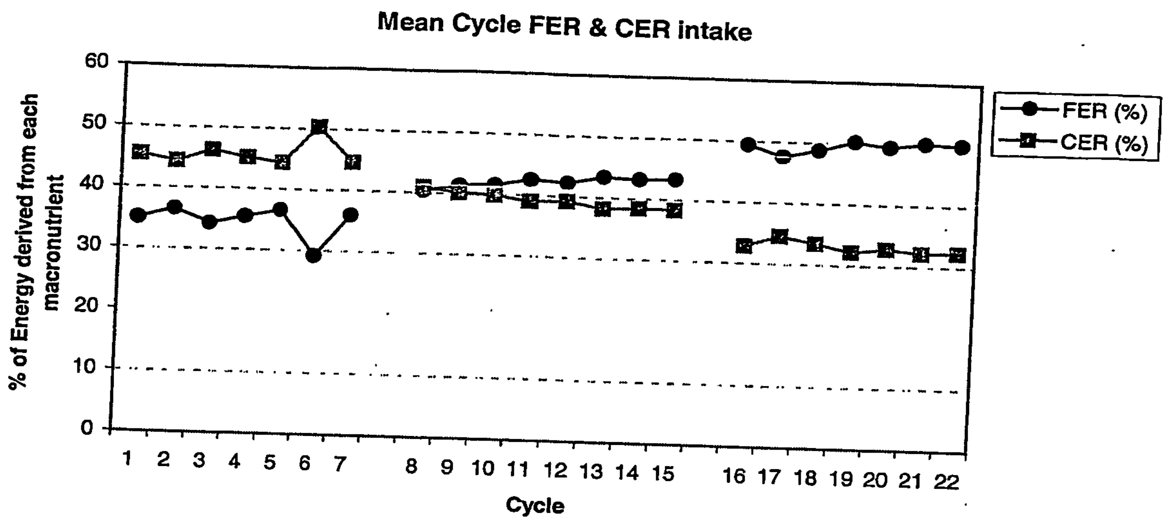


Figure 28: Mean Intakes throughout the trial.

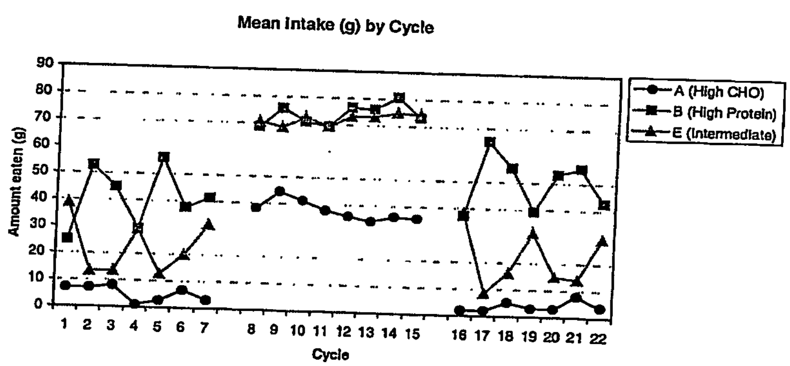
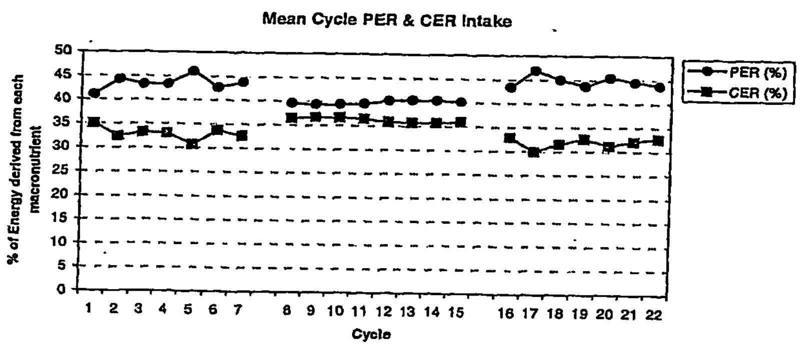


Figure 29:



Mean Cycle PER & CER Intake
% of Energy derived from each macronutrient
50
45
40
35
30
25
20
15
10
5
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
Cycle
PER (%)
CER (%)

This Page is Inserted by IFW Indexing and Scanning Operations and is not part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ BLACK BORDERS
- ☐ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- ☐ FADED TEXT OR DRAWING
- ☑ BLURRED OR ILLEGIBLE TEXT OR DRAWING
- ☐ SKEWED/SLANTED IMAGES
- ☐ COLOR OR BLACK AND WHITE PHOTOGRAPHS
- ☐ GRAY SCALE DOCUMENTS
- ☐ LINES OR MARKS ON ORIGINAL DOCUMENT
- ☐ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
- ☐ OTHER: ______________________

IMAGES ARE BEST AVAILABLE COPY.
As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.